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Novel approaches for effective design of controlled drug release systems, employing hybrid semi-parametric mathematical systems

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"*In God we trust. All others must bring data*."

Brian Joiner, 1985

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Resumo

A libertação controlada de um fármaco do interior de sistemas poliméricos, durante um período de tempo pré-definido é referido como *Controlled Drug Release* (CDR). Um dos maiores desafios para uma libertação continua e reproduzível é a libertação não-intencional de uma grande quantidade de fármaco (*burst*) que ocorre nas primeiras horas/dias de experiência, podendo ter efeitos nocivos para o paciente. O efeito *burst* ocorre com frequência quer com pequenas moléculas, quer com proteínas, quer com micro- e nanopartículas. O *design* de partículas pode, em princípio, ser usado para controlar a quantidade de *burst*, mas até ao momento, nenhum método sistemático está disponível e o *design* de partículas é regido por metodologias empíricas baseada em tentativa e erro. Uma das razões prende-se com o facto que os modelos disponíveis para o *burst* não incluirem explicitamente a relação entre propriedades físico-químicas das partículas no perfil de libertação do fármaco.

Esta tese propõe novas metodologias para o *design* de maneira racional de micro- e nanopartículas de PLGA encapsulando fármacos. Está dividido em três partes principais. Em primeiro lugar, é realizada uma análise quantitativa dos factores físico-químicos que influenciam a quantidade e a cinética do *burst* usando métodos de mínimos quadrados parciais (PLS) e de árvores de decisão. Os fatores com o maior impacto são selecionados para os exercícios de modelação subsequentes. Em seguida, desenvolve-se um modelo híbrido agregado de *bootstrap*, que pode prever com sucesso o *burst* de fármacos de um conjunto independente de experiências de *CDR*. Por fim, um novo método *design* racional é apresentado para a optimização das características de formulação de nanopartículas de PLGA encapsulando proteínas. O método é aplicado com sucesso para optimizar a partícula para proteína "teste", α-quimiotripsina, produzindo um perfil de libertação próximo ao desejado. O método também pode ajudar a avaliar a semelhança da proteína "teste" com uma proteína "alvo" em termos de suas semelhanças no comportamento de liberação de drogas durante o *burst*.

Esta tese propõe o primeiro método racional de *design* de partículas de PLGA que requer apenas especificações do fármaco e do perfil de libertação durante o *burst*. Prevê-se que a aplicação do método reduza significativamente o tempo de desenvolvimento de partículas de PLGA. Com a quantidade crescente de dados de libertação controlada disponíveis, a capacidade preditiva desta metodologia pode ser sistematicamente melhorada, tornando-se uma ferramenta cada vez mais confiável. O método usa uma estratégia de modelação híbrida que descreve o perfil de libertação da droga ao longo do tempo em função das escolhas de *design*, sendo o primeiro do seu tipo na modelação de libertação de fármacos.

Palavras-chave

Libertação controlada, *Burst, PLGA,* nano- and micropartículas, *design racional,* modelação.

Abstract

The controlled release of a drug from a carrier into a medium over a defined period of time is referred to as Controlled Drug Release (CDR). A major challenge for a sustainable and reproducible CDR is the unintentional initial burst, which occurs in the first hours/days of immersion and during which a large amount of drug is released. Also it can have deleterious effects on the host. Burst release happens with both small drug molecules and large proteins and for both drug-loaded PLGA micro- and nanoparticles. Particle design can, in principal, be used to control the amount of burst but no systematic methods are to date available and the design process is governed by trial and error. One reason might be that the available models for burst release do not explicitly account for the particle design parameters.

This thesis proposes novel methodologies that allow for rational design of drug-loaded PLGA micro- and nanoparticles. It is divided in three main parts. Firstly, a quantitative analysis of the physicochemical factors that impact on the amount of burst release and the burst release rate using partial least squares and decision tree methods is performed. The factors with the greatest impact are selected for the subsequent modelling activities. Next, a bootstrap aggregated hybrid model (HM) is developed, which can successfully predict the cumulative drug release of an independent set of CDR experiments. Lastly, a new rational design method is presented for the optimization of the formulation characteristics of protein-loaded PLGA nanoparticles. The method is successfully applied to design the carrier of a mock-protein, α-chymotrypsin, yielding a close to desired release profile. The method can also help to judge upon the similarity of the mock protein with a target protein in terms of their similarities in burst release behavior.

This thesis proposes the first rational PLGA particle design method requiring only the specification of the drug and the desired burst release profile. The application of the method can be expected to significantly reduce the time for PLGA particle development. With the increasing availability of CDR data the predictive power of the method can be further improved towards a systematic and reliable tool. The engine of the method is the hybrid model which links the release profile to the design parameters and is the first of its kind in drug release modeling.

Keywords

Controlled Drug Release, Burst, PLGA, nano- and microparticles, rational design, modeling.

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List of Abbreviations, Symbols and Nomenclature

β	PLS regression coefficient
ϵ	PLS regression residual factor
AIC	Akaike Information Criterion
$AICc$	AIC with second order bias correction
ANN	Artificial neural networks
CDR	Controlled drug release
DCM	Dichloromethane
EMA	European Medicines Agency
FDA	Food and Drug Administration
$F_{B,in}$	Fractional amount of drug release during burst phase
k_b	First order rate constant associated with burst kinetics
MSE	Mean Squared Error
Mw	Molecular weight
PLGA	poly lactic-*co*-glycolic acid
PLS	Partial Least Square
PEG	Poly(ethylene glycol)
PVA	Polyvinyl alcohol
Q	Fractional cumulative amount of drug release
SDS	Sodium dodecyl sulfate
TPGS	d-α-Tocopheryl polyethylene glycol 1000 succinate (vitamin E)
VIP	Variable importance on projection
X	Input matrix for Partial Least Squares
Y	Output vector Partial Least Squares

Chapter 1 - Introduction

1.1. Motivation- Burst in controlled drug release

Drugs do not deliver themselves [1]. A pharmaceutical entity is only as efficient as its carrier. A delivery system is necessary to ensure that a pharmaceutical entity can reach its target. A good delivery system assures that the drug is delivered within pre determined therapeutic rates, and it allows for a sustained and controlled release to ensure therapeutic application, for the duration of the treatment time.

The delivery of a therapeutic agent drug from a delivery system at a specific rate over a determined period of time is referred to as controlled drug release (CDR).

CDR systems have encountered increased pharmaceutical application, because:

i) they allow achieving and maintaining an optimal drug dosage in the body throughout the duration of the treatment, avoiding the undesirable "seesaw" effect of traditional drug administering systems such as tablets [2]. If there are limits of concentrations, i.e., a minimum level of efficacy or a maximum safe concentration, oscillations outside this range (seesaw effect) can result in a waste of material or in concentrations toxic for the host [3];

ii) they have a higher selectivity of a drug to a site;

iii) iii) less frequent dosages are needed, leading to a higher rate of patient compliance [4];

iv) they exhibit lower adverse side effects [2].

v) Also macromolecules, such as peptides and proteins, are very sensitive in terms of stability, and their encapsulation allows protection, especially against gastrointestinal enzymes and pH effects, when administered perorally.

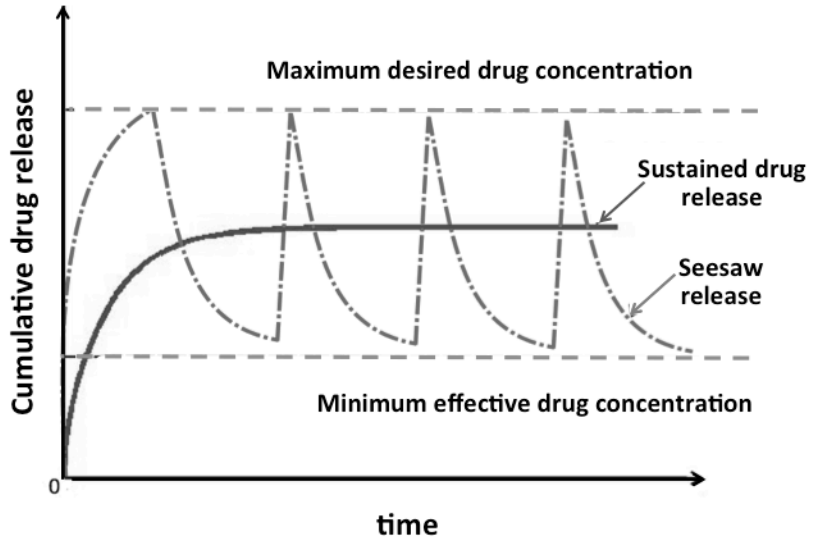

Figure 1-1. Ideal drug release concentration profile achieved with controlled drug release systems (dark blue) compared with drug concentration profile as a result of the intake of series of multiple doses of a traditional drug-delivery system (red).

Hence, the field of therapeutics based on degradable, micro- and nanoparticles, which could allow for a sustained drug delivery in the host, has been booming since the 1980s [5][6].

Nano and micro controlled drug release systems have been studied widely [7], with over 4600 publications in 2016 and this particular type of controlled release systems has found increasing pharmaceutical application.

Miniaturization from micro- to nanoparticles (<1 μm) brought additional advantages [8]. Nanoparticles fall into a size scale, similar to proteins and other large molecular compounds, taking advantages of the membrane transport phenomena naturally occurring [9][10] . The ability to penetrate the cell membranes allows nanoparticles to interact on cell surface receptors. A distinct difference on the behavior of micro- and nanoparticles is that the later have a large surface area to volume ratio leading to more exposed surface and can result in a faster release [11,12].

Therapeutic administration via biodegradable CDR systems also offers a great economical potential. Packaging an existing pharmaceutical entity into a new delivery system extends the formulation patent life. In the 2010 CDR therapeutics were estimated to account for up to 121 billions USD in medical expenses (USA market) [13] a significant increase from only 75 millions USD in 2001. This is thought to represent only a small fraction of the full potential of this technology. It was, for instance, estimated [14] that 90% of the top 100 best-selling prescription drugs could improve their therapeutics capabilities if administrated at a lower rate and less frequent dosages.

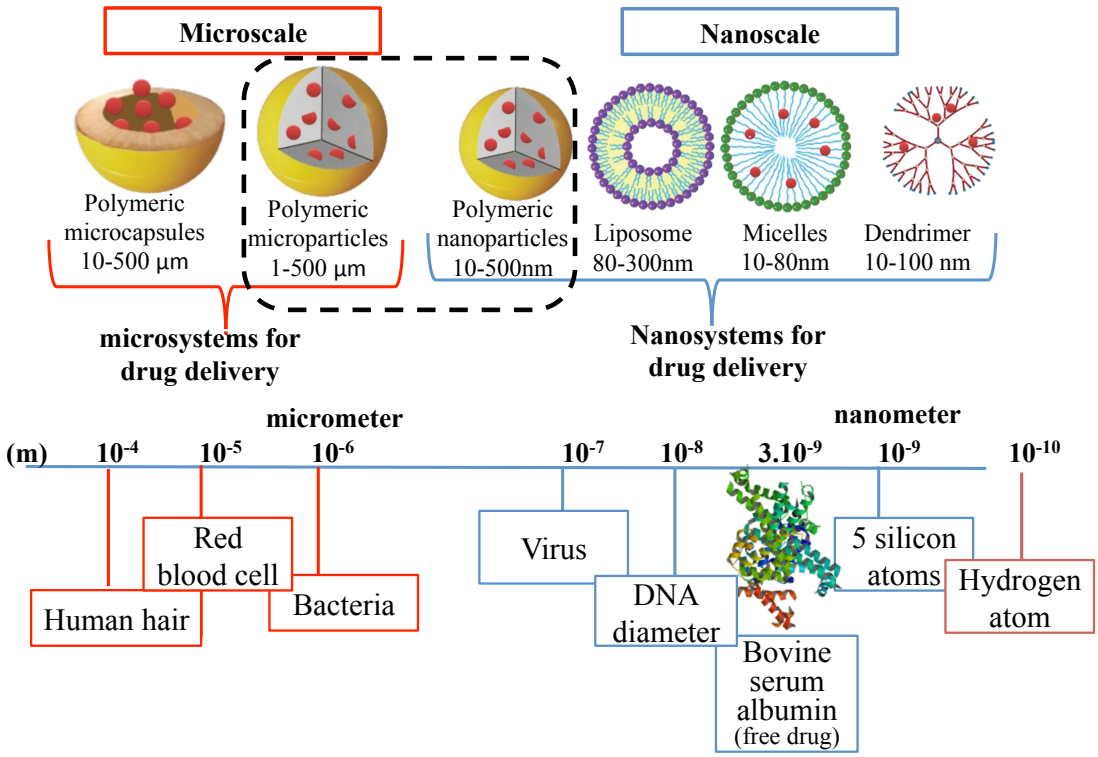

Figure 1-2. Microparticles and nanosystems for drug delivery. A comparison to scale.

Amongst the biodegradable materials developed to formulate micro- and nanocarriers (Figure 1-2) poly lactic-*co*-glycolic acid (PLGA) has shown immense potential [15–17]. PLGA is most popular among the various available biodegradable polymers because:

i) it is biodegradability and biocompatibility;

ii) it has FDA and EMA approval for several drug delivery systems (see Table 2-1);

iii) it has the ability to form systems encapsulating various types of drugs e.g., hydrophilic or hydrophobic small molecules or proteins, utilizing different encapsulating methods [18,19];

iv) it has been extensively studied as delivery vehicle for drugs, proteins and various other macromolecules such as DNA, RNA and peptides [20,21];

v) PLGA systems protect the drug from degradation [22];

vi) it allows for a sustained release [23]; and

vii) it offers the possibility to perform surface modifications enhancing the particle stealthiness and/or better interaction with biological materials [24–28]. Further, PLGA can be tailor made to serve the mechanical properties necessary [29][14]. For more on PLGA surface modification, see section 2.3.

The greatest challenge for a sustained and controlled drug release in PLGA particles, is the typically observed rapid drug release during the first hours of immersion of PLGA particles, referred to as burst [30–37]. The burst release is very frequent in both PLGA micro and nanoparticles, and affects both small drug molecules and large molecules e.g., proteins [38,39] (see Table 2-2 for examples of burst release from PLGA micro and nanoparticles). An intense burst release often leads to a decrease in the time of therapeutic actuation and high initial drug concentrations in the host blood plasma can translate into deleterious effects to the host [38]. Moreover, the burst release reduces predictability and reproducibility, which are prerequisites for a safe controlled drug release system. Consider for instance the case of 40% burst release (common value) of the total drug loading in the first days (Table 2-2) for duration of FDA approved therapeutics with drug-loaded PLGA particles), which can cut short the treatment time by more than 50% of the total possible time (Figure 1-3), making it necessary to resort to extra dosages, such being extremely inefficient at a therapeutic level.

When a typical pack for a one-month course of therapy of drug-loaded PLGA particles is around 1,000-10,000 USD [40,41] (full price for cash paying customers) the burst release is also a large problem from an economic standpoint.

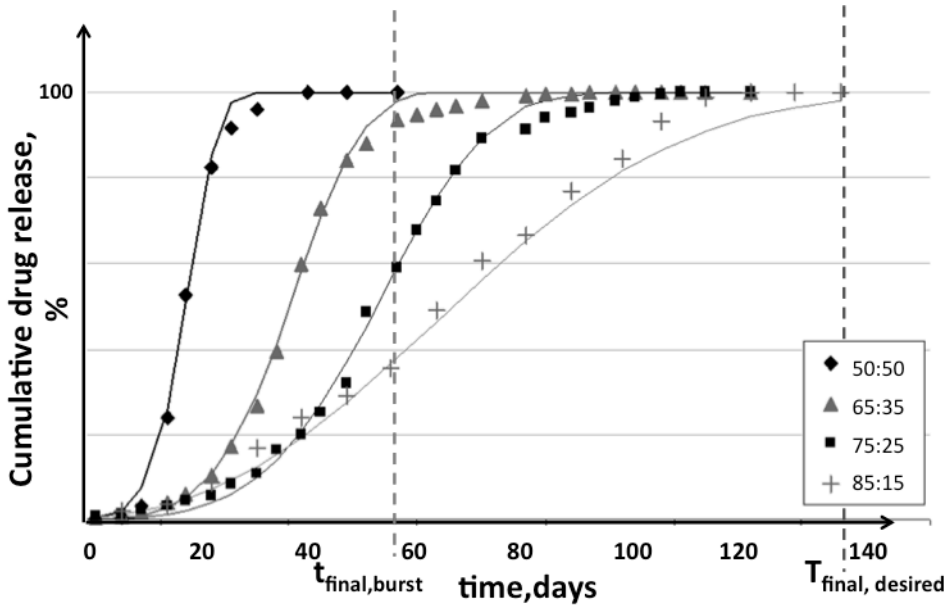

Figure 1-3.Cumulative drug release profiles from PLGA microparticles with different monomer ratios. Particles experiencing an intense burst have a reduced time of actuation. Legend: 85:15 PLGA means 85% of the copolymer is lactic acid and 15% is glycolic acid. Adapted from [42].

Though widely observed, little consensus exist on the mechanisms controlling the burst release. Most mathematical models ignore the burst phase due to lack of mechanistic understanding [38]. The rare cases of burst release being incorporated in release models are approximations to power laws equations, where in order to account for the burst a parameter β is added, which shifts the release profile vertically (equations *11,12*).

Corrigan and Li [43] developed a empirical model describing the drug release from PLGA nanoparticles (equation *8*), identifying two distinct phases, first a burst release, and a second phase where the remaining drug entrapped in the polymer matrix escaped at a different release rate from the burst release. (See section 2.7.2 for more on modeling of burst release.) Still these models are specific for the drug-carrier system they have been developed for, such that for each new system, new release experiments need to be undertaken in order to identify the parameters of the models. Hence, the so far developed mathematical models are of limited usability for the prediction of the drug release of a new drug-carrier system and for the designing of novel more efficient carriers for a specific drug. Thus the design of the optimal biodegradable carrier for a drug is still dominated by heuristics, rules of thumb and a great deal of trial and error experimentation [9,44].

1.2. Thesis objectives

It is the overall aim of this work to develop a model based rational PLGA carrier design framework, in which the model describes the impact of design choices on the control drug release. The work is focused on *in vitro* studies, which are less complex and more reproducible than *in vivo* conditions [45,46]. Several studies that compare *in vivo-in vitro* release experiments and polymer degradation show that the result are comparable [47–49]. The work develops into three main parts:

i. Investigate and quantify the impact factors (molecular physicochemical properties of the drug and carrier system) that control the macroscopic behavior of burst during CDR;
ii. Model the burst release of different drug-loaded PLGA particles as functions of the identified factors; and
iii. Develop a framework that exploits the model to make design choices for new drugs and predefined, desired release profiles.

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1.3. Thesis outline

The remainder of this thesis comprises five chapters.

Chapter 2 provides a brief introduction to controlled drug release (CDR), burst release and CDR modeling. Background information justifying the use of PLGA in controlled release

formulations and a summary of the CDR controlling mechanisms and burst are also overviewed. (Work described in this chapter has been published in a book chapter).

In chapter 3, a quantitative analysis of the factors (drug molecular descriptors, encapsulation method and formulation characteristics) that impact on the amount of burst release and the burst release rate using PLS and decision tree methods is presented. This analysis was utilized as feature selection method choosing the impact factors of the burst release amount and of the kinetics for the subsequent modelling activities (Work described in this chapter has been published in a book chapter and article).

In chapter 4 drug release is modeled via the Corrigan equation in which changes in its parameters associated with the total amount of drug released during burst and the burst kinetics are described by artificial neural networks (ANNs). The ANNs are derived as functions of the synthesis parameters and molecular descriptors of the drug identified by the decision tree modeling. A bootstrap aggregating identification strategy is used for the development of the hybrid model. The model is used to predict the cumulative drug release of an independent set of CDR experiments for testing its capabilities. (Work described in this chapter has been submitted for publication.)

The bootstrap aggregated hybrid model developed (in chapter 4), is used in chapter 5 for predicting the changes in the initial burst release of two proteins when manipulating the formulation characteristics of the encapsulating particles to achieve a desired profile.

A rational particle design approach is developed that manipulates the formulation characteristics to match a desired release profile while also minimizing the deviations between the predictions of the aggregated models. The proposed method is applied to a mock protein (α-chymotrypsin), which exhibits similarities with an expensive drug, Activin A, in terms of important molecular descriptors with the target drug. A sensitivity analysis is performed at the optimal formulation characteristics to assess the sensitivity of the cumulative drug release to changes in the formulation characteristics.

Finally, in chapter 6, the main accomplishments achieved in this thesis are presented and summarily possible improvements of the investigated strategies and suggestions for future work are detailed.

1.4. Thesis main achievements

In this thesis the impact of physicochemical factors, such as drug molecular descriptors, encapsulation method and formulation characteristics, on the amount of burst release and the burst release rate are quantified using PLS and decision tree methods. An analysis utilizing both methods to identify the most impactful input factors on the amount of burst release and respective kinetics and the findings are in mainly consistent.

Due to a much better performance of decision tree models, these are utilized as feature selection method to pinpoint the impact factors for the burst release.

Subsequently a bootstrap hybrid model methodology was derived for the prediction of cumulative burst drug release. The aggregated hybrid model comprises an empirical burst release equation in which variations in its parameters associated with the total amount of drug released during burst and the burst kinetics are described by artificial neural networks (ANNs). The aggregated model was used to predict the cumulative drug release of an independent set of CDR experiments having good agreement between the predicted and the experimentally measured cumulative drug release profiles been achieved.

Finally, the bootstrap aggregated hybrid model developed was utilized in an optimization framework to design protein-loaded PLGA particles such that a predefined, desired release profile is obtained, while also showing low standard deviations between the predictions of the aggregated models. The proposed rational design method was successfully applied to a mock protein (α-chymotrypsin), which exhibits similarities with an expensive drug, Activin A, in terms of important molecular descriptors. A sensitivity analysis was performed for the optimal formulation characteristics and the results, when compared to literature findings, showed good agreement. The burst release predictions of the mock protein were then compared to those obtained for Activin A using the same mock-protein optimized particle design. Good agreement between the predicted releases for both protein-carrier systems was achieved with equal formulation characteristics.

Also the use of a mock-protein showed to be an important tool to aid the discovery of the working space of acceptable formulation characteristics, given that a good drug representative mock candidate is found.

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Chapter 2 - PLGA, Burst and Controlled Drug Release Modeling: An introduction

2.1. Introduction to Controlled release

Pharmaceutical products can be administered to the human body via various pharmaceutical dosage forms, like tablets. In order to maintain a therapeutic level of the drug in the host most systems require frequent and repeated doses. This can yield an undesirable ‘‘seesaw’’ effect of the drug level in the host blood plasma (Figure 1-1), where oscillations outside maximum safe concentrations and minimum effective dosage can result in a waste of the valuable drug or in concentrations toxic for the host [1]. The introduction of controlled release therapies based on biodegradable microparticles has revolutionized the field of drug release. They have been widely studied for the last 3 decades [2,3]. Special interest has been focused on biopolymers with long degradation times, which allow for i) a non-invasive administration, without the need of extraction, as opposed to implants and ii) for sustained and controlled release throughout the length of the treatment, which can reach several weeks or months (Table 2-1).

The advent of miniaturization technologies (<1 μm) introduced nano-particulate systems with additional advantages. Nanoparticles fall into a size scale similar to proteins, being able to penetrate cell membranes [4,5]. Also, the delivery of anticancer agents, e.g., paclitaxel, with microparticles was very slow due to the drug poor solubility and diffusivity. The transition to encapsulating paclitaxel in nanoparticles instead of microparticles increased the surface area available for drug diffusion. Though the nanoparticles polymer matrix is more compact (leading to a slower diffusivity in the polymer matrix [6]), the overall effect results in an increase in the drug release rate with a decrease in particle size when compared with microparticles of the same polymer [7].

Controlled release systems have been designed to enable a sustained drug release over time, to protect the drug from premature elimination and to assist drug in crossing physiological barriers[8]. By augmenting the duration of one course of treatment, the patient compliance increased significantly. Controlled release systems add commercial value to existing pharmaceutical entities; i) the repackaging of an existing drug in a controlled release carrier can extend the patent protection time and ii) a recent study, estimated [9] that 90% of the top 100 best-selling prescription drugs could improve their therapeutics capabilities if administrated at a lower rate and less frequent dosages.

2.2. The importance of PLGA

Poly (Lactide-co-Glycolide) acid (PLGA), a synthetic polyesters copolymer, is composed by different ratios of Poly(lactic acid) (PLA) and Poly(glycolic acid) (PGA) [10] (see Figure 2-1). When the ratio of PLA is 3 to 1 of PGA, the resulting PLGA polymer is referred to as PLGA (75:25).

(a) (b)

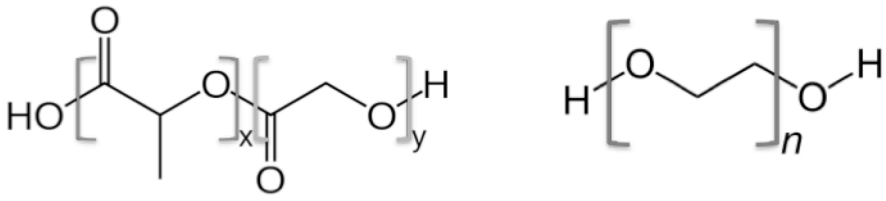

Figure 2-1. (a) Structure of PLGA; x= lactic acid units, y=glycolic acid units; (b) Structure of Polyethylene glycol (PEG); n= number of monomers

Controlled delivery systems based on PLGA have a tremendous interest due to an array of properties. PLGA is biocompatible and the biodegradable [11–13]; this polyester is degraded by hydrolysis. The resulting products are either excreted by the kidneys or eliminated as water and carbon dioxide via established pathways [13].

The manipulation of PLGA polymeric chain (PLA/PGA ratio, PLGA molecular weight) allows for a fine-tuning of the mechanical properties of PLGA particles [11,14,15]. PLGA micro and nanoparticles can be synthesized in an array of different size ranges and with mechanical properties (e.g., degradation rate) to suit the needs of the drug delivery. PLGA particles have been approved by the FDA in several PLGA-based drug products (Table 2-1) [16] and they are widely used as controlled delivery carriers [8,17].

Several reviews on the usage of PLGA based micro and nanosystems can be found in [17–19]. In this study, PLGA micro- and nanoparticles are studied because i) due to their widespread use significant amounts of data are available; ii) choices in the design of the particles have a significant impact in the drug delivery profile; iii) due to the expected high use in the future, a model that allows for rational design of the particles will provide a significant contribution.

In addition, PLGA particles with a surface transformation, a PEG coating were included in the study as described in section 2.3.

Table 2-1. FDA-Approved Drug Delivery Products Using PLGA Polymers and Under Development

Approval Date	Product name	Active Ingredient	Strength	Indication
1984	Vivitrol	Naltrexone	380 mg, 4 weeks	Alcohol dependence

	1988	Suprefact Depot	Buserelin acetate	1 mg, 8 to 12 weeks	Prostate cancer
	1989	Zoladex	Groserelin acetate	3.6 mg 4weeks	Prostate and breast cancer
	1989	Lupron Depot	Leuprolide acetate	7.5 mg, 1 month	Prostate cancer (palliative treatment)
	1993			7.5 mg, 1 month	Central precocious puberty
	1995			3.75 mg, 1 month	Endometriosis
	1998	Sandostatin LAR	Octreotide acetate	10-30 mg, 4 weeks	Acromegaly
	1998	Atridox	Doxycycline hyclate	50 mg / 1 week	Periodontal disease
	1999	Nutropin Depot	Recombinant human growth hormone	2.25 mg/kg, 2 weeks	Growth hormone deficiency
	2000	Trelstar	triptorelin pamoatea	3.75 mg, 1 month	Advanced prostate cancer
	2001	Arestin	minocycline	1mg / *	Adult periodontitis
	2002	Eligard	leuprolide acetate	3.5 mg, 1 month	Advanced prostate cancer
	2003	Risperdal Consta	risperidone	12.5, 2 weeks	Schizophrenia & bipolar I Disorder
	2006	Vivitrol	naltrexone	380mg, 1 month	Alcohol dependence & opioid dependence
	2007	Somatuline	lanreotide	120mg/*	Acromegaly
	2009	Ozurdex	dexamethasone	0.7mg/ *	Macular edema
	2012	Bydureon	Exenatide	2mg /1 week	Diabetes (glycemic control)
	2012	Lupaneta Pack	Leuprolide and norethindrone acetate	3.75mg / 1month	Endometriosis
	2014	Signifor LAR	Pasireotide parnoate	40mg/ 4 weeks	Acromegaly
CF2		Oncogel	Paclitaxel	PEG-PLGA-PEG **	Solid tumors
CF3		Sanvar® SR	Vapreotide	**	Esophageal bleeding varices (EVB)

* Varying dosage; ** Not specified, CF2= Clinical trial; Phase III, CF3= Clinical trial; Phase III.

2.3. PEGylation of particles

In vivo use of PLGA particles presents several challenges as for instance; i) they have poor stability in water [20], ii) blood proteins are easily adsorbed on their surface and promote opsonization, leading to aggregation and rapid clearance from the bloodstream [20–22] and iii) particles larger than 200nm are easily removed by the liver and spleen, thus reducing the circulation time and the concentration of the drug [23].

Some of these difficulties can sometimes be overcome by turning the particle "invisible". As Allan [24] wrote, "if you want to be invisible, look like water". Hence, coating the surface of nano- and microparticles with polyethylene glycol (PEG), a hydrophilic inert polymer, will offer hydrophilic properties to the PLGA particles. This surface coating "masks" the PLGA particles, inhibiting their recognition by the immune system (opsonization) and avoiding reticuloendothelial system, which can result into, PEGylated PLGA particles having a longer half-lives in the blood [23,25–28]. Also, this surface transformation is shown to not compromise the activity of the therapeutic agent significantly [26].

The extent of stealth qualities of PEG has been put in question by several researchers. Several *in vivo* studies have observed that a small amount (2.5-10%) of PEG-coated particles were removed by the spleen and liver 1 hour after administration [29–31]. Fortunately this quantity was observed to decrease with the increase of concentration of PEG-coated particles [29]. It is yet unknown if the opsonization of PEG-coated particles is targeting the PEG vesicles or an exposed (non-coated) area of the particle [32–35]. Still, PEG-coated particles were found to have long circulation times after extensive opsonization [36].

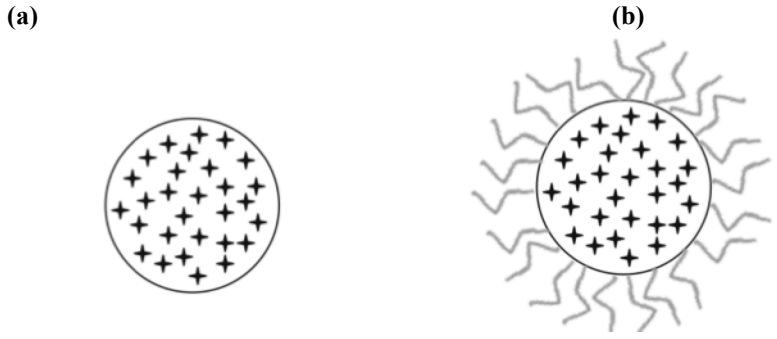

Figure 2-2. Schematic (a) drug-loaded PLGA particle; (b) PEGylated PLGA nanoparticle.

Nevertheless, PEG is the most widely used "stealth" polymer in controlled drug delivery, due to its long history of safety in humans and gained classification as it is "Generally Regarded as Safe" (GRAS) by the FDA [37,38]. Reviews on the applications of PEGylation of PLGA

particles can be found in[23,35,37,39–41].

2.3.1. Drawbacks of PEGylation

Despite the advantages stated, PEGylation of particles can influence the rate of drug release. Hence in this work PEGylated PLGA particles were also considered. For instance, Daravan et al. [42] showed that drug release from PLGA-PEG particles suffered an abrupt initial burst with a large amount of drug released immediately upon hydration. Several researchers have observed an increase in the initial burst when dealing with PEGylated PLGA particles, versus their PEG free counterparts [43–49]. This might be due to its high hydrophilicity, whereby PEG-PLGA particles have an increased initial water hydration, which in turns accelerates the release of the drug adsorbed at the particle surface [50].

2.4. Release mechanism related to the CDR from PLGA particles

A great number of phenomena, i.e., true release mechanisms occur during the drug release from PLGA particles, (Figure 2-3). However, certain assumptions can be made to simplify the understanding of the drug transport [51]. The phenomena, which actually control the rate of the drug release, are often referred to as rate-controlling release mechanisms. For the modeling of the CDR (see section 2.5), the determination of the rate-controlling mechanisms among all the "true" release mechanisms is often a pre-requisite [52–59].

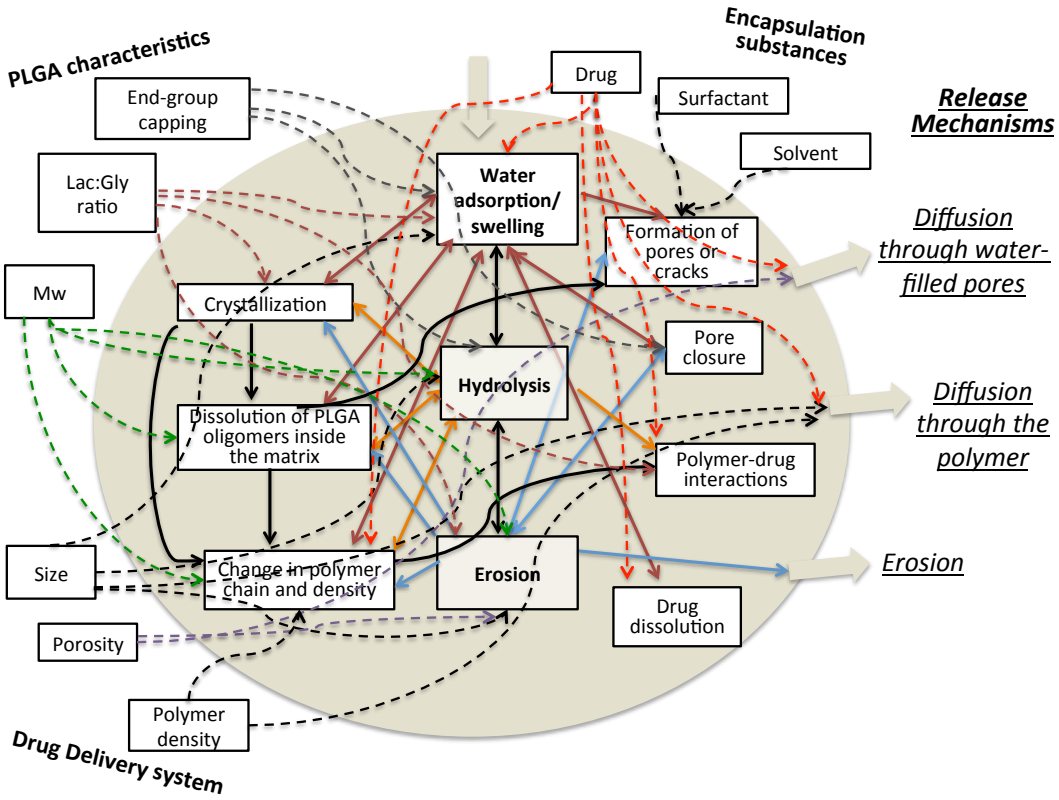

Figure 2-3. A scheme of physicochemical processes taking place within PLGA matrices, leading to drug release. The influence of processes on drug release and on other processes is illustrated by arrows. Note that some arrows point in both directions. Adapted from [52].

Degradable delivery systems can be designed to exploit a variety of mechanisms. Langer [57] suggested to categorize the delivery systems into three main rate-controlling mechanisms in CDR; diffusion-, erosion-and swelling-controlled, which has been widely adopted [51,52,55,6,60]. The limiting step in the release of majority of types of drug-embedded nanoparticles is the drug diffusion through water or biological medium filled pores (hereinafter referred to *diffusion* for simplicity) [51,6,61–66]. Fredenberg [67] explained the dominance of diffusion versus other true phenomena as follows: (i) proteins or peptides, are often too large and too hydrophilic to diffuse through the polymer; and (ii) drug transport is often faster than polymer erosion. Even in cases where erosion plays an important role, diffusion is used to describe the first stage of the release [55,68,69]. Depending on the physicochemical properties of the particles, the release is either purely fickian (Figure 2-4, blue line), where the diffusion is the rate-controlling mechanism, or the release can have an apparent "S" shape. The "S" shape profile is marked by an initial diffusion phase, followed by a lag phase where a low amount of drug is released and finally a second rapid drug release phase, where usually the remaining drug is diffused via an erosion-induced porous matrix (Figure 2-4, orange line) [70,71].

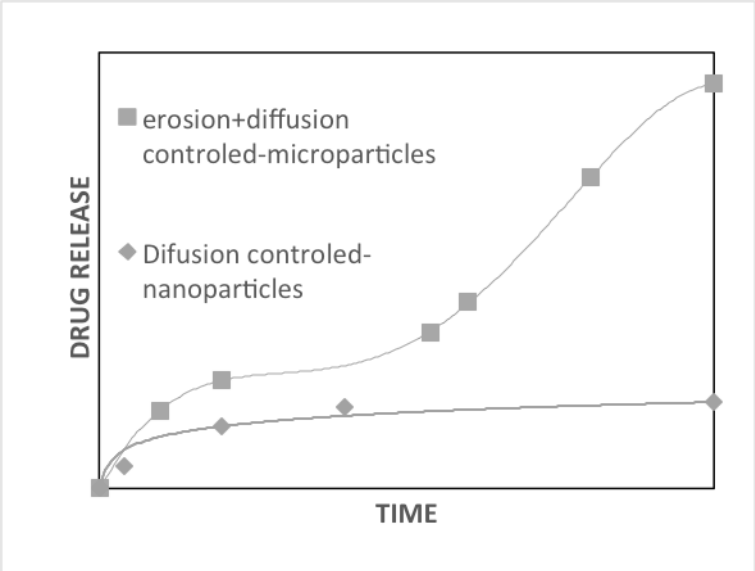

Figure 2-4. Model of Heller and Baker [72] **describing drug release from thin biodegradable polymers particles undergoing bulk erosion (squares), and purely diffusion-controlled release kinetics calculated using the classical Higuchi model** [73] **(diamonds). Adapted from** [51]**.**

2.5. Influences of particle design and synthesis choices on CDR

The degree to which the phenomena shown in Figure 2-3 control CDR rates depends on various properties of the drug-carrier system. For instance, the manipulation of the PLGA polymeric chain (PLA/PGA ratio, PLGA Mw) is a known and important parameter to fine-tune the CDR [15,74]. Other variables are the size of the carrier, concentration and solubility of the drug in the polymer and medium as well as encapsulating membrane diffusion [51,52,75–77]. Studies of the impact of several synthesis parameters and drug properties on the drug release profile can be found in [71,78–85].

Rothstein et al. [86] reviewed the literature and gathered the impact of several variables that impact the CDR. However they only considered the impact of one variable at a time and neglected the interdependencies between variables [87]. In addition the impact of the variables is not quantified.

2.6. Burst

The high surface area to volume ratio of nanoparticles contributes to a rapid drug release from the PLGA polymeric matrix. This is referred to as burst release.

A rapid release of drug can occur during the first hours of immersion of a controlled drug release (CDR) system [10-16]. This burst release is likely due to leakage of undissolved drug particles located near the particles surface.

Also a poorly entrapped drug easily diffuses out of the carrier during the initial contact with the biological fluid, giving rise to a strong burst. In a few particular cases it can be designed as a triggered burst [17], but usually it is an uncontrolled, undesired phenomenon (see e.g., review of Huang et al. [18]). An intense burst drug release often leads to a shorter total drug release time and high initial drug concentrations that can have deleterious effects on the host.

Very high burst release was observed by several researchers. In many cases up to 80% of the total drug loading was released within the first 24 hours [80]. Moreover, the burst release hinders predictability and reproducibility, which are prerequisites for a safe controlled drug release system. Some researchers stated that for there to be an initial burst, it is necessary to have poorly encapsulated drug adsorbed at the particles surface [74]. Hence, a reduction of the initial burst is achieved by an optimization of the encapsulation efficiency, which is quite difficult to control especially during the solidification phase of the particles synthesis [87]. Other strategies that are at least to some degree consensually accepted exist in regards to how to decrease the burst release. These strategies aim at manipulating particular physicochemical properties of the PLGA carrier [53,87]. Although well documented, no consensus exists on the underlying physical mechanisms neither on how to prevent mechanisms that result in a burst release [53] .

Table 2-2. Examples of burst release from PLGA micro and nanoparticles observed in published work.

Released drug	Molecular weight, Da	PLGA (Lac:Glyc), / Mw (kDa)	Amount of burst drug release, %	Source
Blue dextran (model drug)	2000	75:25 / 5	30	[88]
BSA	66000	75:25 / 40	67.5	[81]
Lysozyme	~ 14400	50:50/ 27	50	[89]
BSA	66000	50:50 /*	60	[78]
BSA	66000	PLGA(75:25) – PEG / 45	37.5	[90]
FITC-BSA	66000	75:25 / 14	70	[91]
Ciprofloxacin	331.35	50:50 / 46	40	[92]

Dexamethasone	392.46	50:50 / 55	60	[71]
5-fluorouracil	130.08	85:15 / 13	80	[80]

* Not specified by the authors

2.7. CDR modeling for PLGA particles

The majority of PLGA carriers can be designed to exploit either diffusion-controlled, swelling/erosion-controlled, or combinations of all mechanisms [52,57,6]. Mathematical models play an important role in the design (or optimization) of new controlled delivery carriers. A variety of carrier systems have been studied by establishing both simple and complex mathematical models.

The first to formulate a mathematical model for controlled drug delivery was Higuchi [93] in 1961. The model describes the rate of release of a drug suspended in ointment bases into a medium in perfect sink conditions (Figure 2-5).

The general form of the Higuchi model is $Q = k \cdot \sqrt{t}$ with $= \sqrt{2 \cdot C_{in} \cdot C_s \cdot D}$, where Q is amount of drug released at time t, C_{in} is the drug concentration, C_s is the solubility of the drug and D is the drug diffusion constant in the medium.

The amount of drug released correlates with the square root of time.

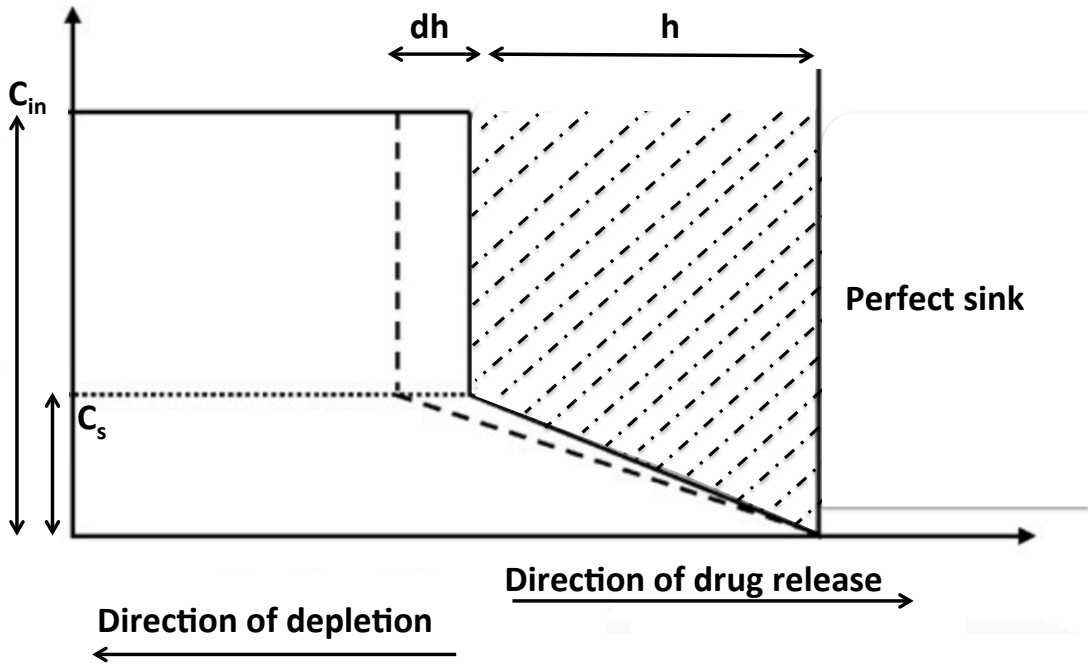

Figure 2-5. Theoretical drug concentration profile in an ointment, in contact with a medium in perfect sink conditions at time t (solid line) and at time t+dt (dashed line). Variables h represents the distance of the wave front, separating three different concentrations in two boundaries (right to left) ointment free of non-dissolved drug excess (sink conditions) with ointment with non-dissolved drug excess and at left from the ointment interface at time t. dh is the distance the wave moves inwards during the time interval *dt*.. [Adapted from [93,94]**.**

The Higuchi model can be derived to fit other types of morphologies, but it can easily be misused neglecting the assumption used to derive the model [93], so much so that Siepmann et al. wrote an article on the use and misuse of the Higuchi model [94].

After Higuchi, several researchers have developed models of drug release from micro- and nanoparticles based on a mechanistic or phenomenological interpretation (e.g., [19-26]).

An extensive review of modeling of drug release from microspheres can be found in Arifin et al. [6]. The authors structure the review into models dealing with i) diffusion-controlled systems where the drug diffuses through the non-degraded polymer matrix; ii) swelling-controlled systems where the diffusing of the drug in enhanced due to polymer swelling, and iii) erosion-controlled systems, where the drug is released due to the polymer matrix degradation. The first case, purely diffusion-controlled systems, deals with rigid, non-degradable particles, typically reservoir-like. The second Fick´s equation of diffusion can be used to describe these systems ((Table 2-3, equation *1*) [95]). Although PLGA particles are degradable, several researchers have "borrowed" non-degradable mathematical models for the

release from PLGA particles under the assumption that the particle radius is constant, and managed a good fit at least for "small" times (Table 2-3, equations *2* and *3*). The second and third categories represent the release from degradable particles, the category PLGA carriers belongs to. Mechanistic and empirical models from these categories are shown in Table 2-3 (equations *6*,*67*,*9*,*10* and *11*). Finally Arifin et al. delved into the applicability of using the existing CDR models from microparticles models to nanoscale drug delivery systems, specifically focusing in the challenges of hydrophobic and hydrophilic molecules delivery.

Table 2-3. Mathematical models describing drug release from spherical degradable micro and nanoparticles.

	Controlled drug release model*	Controlling mechanisms / Notes	Source
	Mechanistic		
(1)	$\frac{Q_t}{t} = 4\pi \frac{RR_i}{R - R_i} DC_i$	Diffusion / reservoir	[95]
(2)	$Q = 6\sqrt{\frac{Dt}{\pi R^2}} - \frac{3Dt}{R^2} \quad for\ Q < 0.4$	Diffusion	[96]
(3)	$Q = 1 - \frac{6}{\pi^2} exp\left(-\frac{\pi^2 Dt}{R^2}\right) \quad for\ Q > 0.6$	Diffusion	[96]
(4)	$Q = 4\sqrt{\frac{D_e t}{\pi R^2}} - 3\frac{D_e t}{R^2} + F_E\left(\frac{e^{k_{degr}(t-t_{max})}}{1 + e^{k_{degr}(t-t_{max})}}\right)$	Erosion	[97]
(5)	$Q = 1 - exp\left[\frac{-(t - t_{lag})^b}{t_{scale}}\right]$	Erosion	[98]
(6)	$Q = 1 - \left[1 - \frac{k_{ero}}{C_0 R}\right]^3$	Erosion	[99,100]
(7)	$Q = 1 - \left[1 - \frac{k_{ero}}{C_0 R}(t - t_{lag})\right]^3$	Erosion + lag	[101]
(8)	$Q = F_{Bin}\left(1 - e^{k_b t}\right) + (1 - F_{Bin})\left(\frac{e^{k_{degr}(t-t_{max})}}{1 + e^{k_{degr}(t-t_{max})}}\right)$	Erosion - burst	[102]
	Empirical		
(9)	$Q = kt^n + \beta$	Swelling	[103]

(10)	$Q = k_1 t^m + k_2 t^{2m}$	Swelling	[104]
(*11*)	$Q = k(t - t_{lag})^n + \beta$	Swelling + burst	[105]

Q(t)=cumulative release

Legend: R=r=particle radius, R_i=core radius, D=Diffusion coefficient, D_e= approximated drug diffusivity, k_{deg}= surface degradation rate constant, F_e= fractional amount of drug release during erosion, t_{max}= time to maximum matrix erosion rate, t_{lag}= lag time before the polymer matrix erosion, k_{eros}= surface erosion rate constant, C_0 = initial concentration of the drug in the matrix, $F_{bin=} \beta$ = fractional amount of drug release during burst, k_b=burst kinetics parameter

Another model shown in Table 2-3 is the Corrigan model equation *8* [102]. It features the controlling steps of an initial diffusion and a second phase where the drug entrapped in the polymer is released, due to polymer erosion. Due to its ability of modeling the initial burst followed by a steadier release and its simplicity, the Corrigan model will be utilized in this work. For more details, see section 2.6.2.

Siepmann and Göpferich [51] wrote a review on "Mathematical modeling of bioerodible, polymeric drug delivery systems" the category where PLGA particles fall into. The authors analyzed models based on phenomenological events, diffusion phenomenon (i.e., Fick equation derivations) and chemical reaction theory. As noted by the authors, there is a panoply of mathematical models, that due to the significant chemical and physicochemical differences among biodegradable systems are not usually able of being utilized for other carriers. Most models that were developed to describe a CDR system are only valid for the specific carrier-drug system for which they were developed.

An interesting approach carried out by Siepmann et al. [106] where a Monte Carlo sampling was combined with a mechanistic model. The mechanistic model takes in consideration the possible controlling mechanisms in CDR from erodible microparticles (diffusion, dissolution, erosion of the polymer and moving boundary conditions). Initial conditions are defined regarding the porosity of the polymer, and how the matrix porosity (i.e., if a certain area, *dA*, of the three-dimensional particle is occupied by drug, polymer or pore) evolves along time. The model fitted well one CDR experiment with an "S" shape, where several physical processes were involved at different stages of the release. Although a good agreement was achieved with experimental data, like other mechanistic models, the impact of physicochemical properties of the drug and polymer matrix are not described. Hence it would have limited value for the design of carrier system.

2.7.1. Parameter Identification

For a model to be able to accurately describe the drug release, it is necessary to identify the model parameters (e.g., diffusion and erosion coefficients), by means of carrying out a

number of experiments. These parameters are specific for a given drug-polymer matrix combination. This means that when designing a new drug carrier, these models will be of little use, i.e., its predictability capacities are limited to the drug-particle combination for which its parameters were identified.

Also, the experimental conditions and drug properties, which have a significant impact on the controlled release profile, are at most only implicitly taken into account by these models. More specifically, the model parameters that characterize the drug release profile are identified from data and their values are context dependent implying that for new drugs and experimental conditions the parameters are per se unknown. The average carrier radius is typically included in such models, enabling the study the impact of the carrier size on the CDR profile. However, the carrier chemical composition (e.g., monomers ratio), which has a significant impact in the CDR profile [74], is indirectly represented by empirical model parameters and cannot be extrapolated to a different context. Thus these models are often unfit to predict the effect neither of the carrier chemical composition, nor of the experimental conditions, formulation characteristics or drug chemical properties on the CDR profile.

Data driven approaches have also been used for CDR modeling and analysis. These methods can be used to identify significant correlations between macroscopic kinetic parameters and physiochemical properties of the carrier and/or drug without a formal mechanistic interpretation. Matero et al. [107] divided the cumulative drug profile into seemingly linear phases and then applied Partial Least Square regression (PLS) to each phase individually, using molecular descriptors as inputs. The final PLS models were however specific to the type of hydrophobic matrix tablet studied.

Utilizing molecular descriptors allows for an incorporation of a huge amount of information of the drug molecule into the release model (not a mechanistic model, but still), until then unprecedented. Todeschini et al. [108] defined MDs as:"… the final result of a logic and mathematical procedure which transforms chemical information encoded within a symbolic representation of a molecule into a useful number". MDs have been used to describe physiochemical properties of potential candidates in the process of drug screening, as for instance to check the levels of potential toxicity, hydrophilicity, lipophilicity and to check the overall drug likeness of the drug candidate and the host medium [109–112]. Still, very little research is reported regarding MDs in CDR, especially in micro- and nanoparticles. Work reporting the use of MDs to describe effects on a macromolecular scale in CDR has been done by Szlęk et al. and Zawbaa et al. [63,113,114]. The authors used the formulation characteristics and time in addition to physiochemical properties of the drug (molecular descriptors), as inputs to a number of data-driven approaches (genetic algorithms, artificial

neural networks, random forests, multivariate adaptive regression splines, etc.) for modeling the cumulative amount of drug released from PLGA microparticles. While the drug release profiles were fairly well modeled, understanding how the formulation characteristics or drug properties impact on the drug release, i.e.,on the amount of drug released or the release kinetics was not straightforward due to the empirical nature of the models.

2.7.2. How to model burst

Though burst is a frequent event, most mathematical models ignore the burst phase due to lack of mechanistic understanding. Huang and Brazel [53], in a rare review on the burst mechanisms that control drug delivery noted that a simple approximation could be made to power law models (equation *9* and *11*), by adding a parameter β, which shifts the drug release profile vertically by a certain amount (Figure 2-6).

The parameters β, k, t_{lag} and n were determined after fitting the equations with experimental data.

The authors also cited the rare attempts made into modeling mildly intense burst releases in swelling controlled systems using purely empirical equations [115,116].

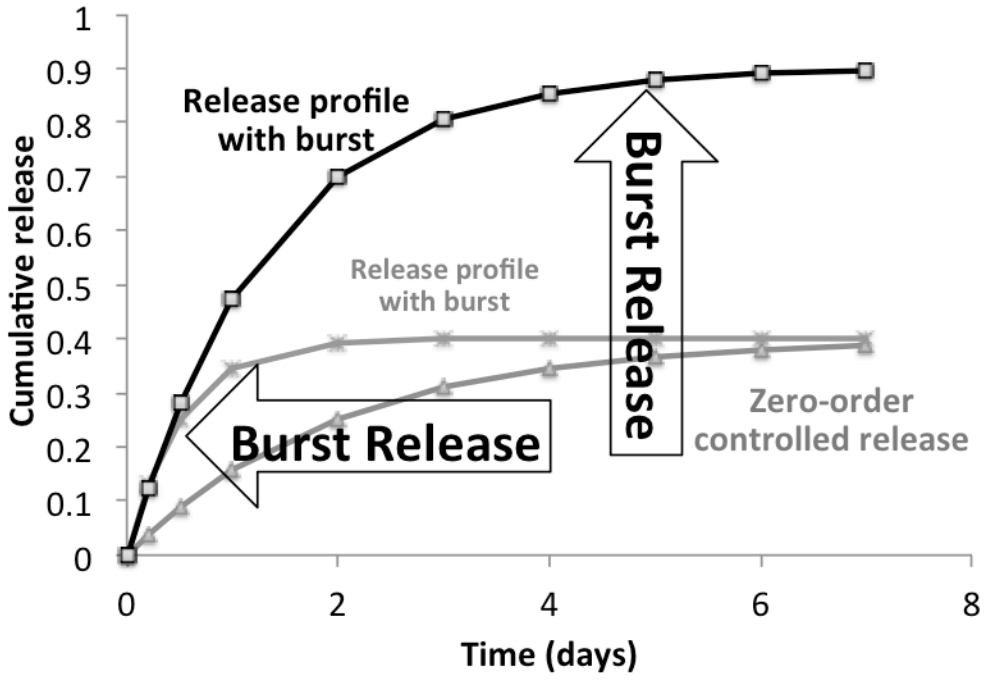

Figure 2-6. Schematic representation of the ideal controlled drug release compared with initial burst release.

Corrigan and Li [102][117] developed a model describing the release from both small and macromolecules from PLGA nanoparticles (equation *8*). The model identified two distinct release phases: a first stage which is the rapid dissolution (diffusion controlled), of the drug in the particle surface into medium and the second phase, describing the release of drug entrapped in the polymer. This second phase is controlled by polymer-matrix degradation. In this model, the first term refers to the burst phase release profile

$$Q_b = F_{bin}(1 - exp^{-k_b t}) \qquad (12)$$

where F_{bin} is the burst fraction at the "final" time of burst, and k_b is a first-order constant associated with the rate of 'burst' release. The rate constant k_b is equal to $D \cdot A \cdot C_S/W_L \cdot F_{Bin} \cdot h_1$, where D and C_S are the diffusion coefficient and solubility of the drug, respectively, A is the surface area of drug available for dissolution, h_1 is the apparent aqueous diffusion boundary layer thickness and W_L is the total drug loading. Again, the parameters F_{bin} and k_b can be identified by means of carrying new controlled release experiments for each different carrier system. The Corrigan model fits the experimental data quite well, but is very time laborious to use it to predict new drug-carrier profiles.

Donaldson et al. [118] used a similar overall model, in which the term referent to the burst is equal to the one proposed by Corrigan and Li [102].

As explained in section 2.6, the models described above are only valid for the specific carrier-drug system for which they were developed. This means that when designing a new drug carrier, these models will not be able to accurately describe the drug release because the model parameters (e.g., diffusion and erosion coefficients, available surface area) are specific for a the drug-polymer matrix combination, and these parameters have to be re-identified for a new carrier system by means of carrying out new experiments.

In this work, a novel approach was utilized, where the burst release from drug-loaded PLGA particles was modeled utilizing an array of data-driven methodologies combined with the burst release model proposed by Corrigan et al. [30] (equation *12*). The combined models describe the changes on the amount of drug released (F_{bin}) and kinetics of burst (k_b) in function of synthesis, particle design and the drug molecular properties.

Finally the bootstrap aggregated hybrid model developed (in Chapter 4) was exploited in a rational optimization approach for optimal design of the formulation characteristics of a given drug by manipulating the formulation characteristics of the particle design to achieve the desired profile.

2.8. Hybrid modeling

As noted above (section 2.7), data-driven empirical and mechanistic modeling approaches have been applied for the controlled drug release modeling. In this thesis, hybrid models are developed that combine the mechanistic knowledge with data-driven techniques. This is a well studied modeling approach in process systems engineering [119], that combines process knowledge with data-driven techniques.

2.8.1. Basic hybrid modeling structures

Hybrid models are typically represented by a combination of white and black boxes, representing the mechanistic knowledge and the data-driven model, respectively. These two boxes can be arranged in two ways, in serial and parallel (Figure 2-7). In serial data-driven – mechanistic structures, the data-driven block calculates intermediate process variables that are feedforwarded to a mechanistic model or vice-versa. Serial structures arise when knowledge is missing for a particular part of the process, being the job of the data-driven model to identify only the missing part. Such happens in the case investigated in this thesis, where the parameters of a mechanistic equation describing the burst release will be first identified by a data-driven model (Figure 2-7a). This serial approach allows describing a system, even though the underlying phenomena are unidentified at the starting point. On the contrary, in parallel hybrid model structures there are no gaps in the mechanistic description but the problem is that the mechanistic model does not perform sufficiently. The data-driven model is placed in parallel to the mechanistic block and set to identify patterns from the mechanistic model residuals. In other words, the data-driven model is asked to correct the mechanistic model predictions.

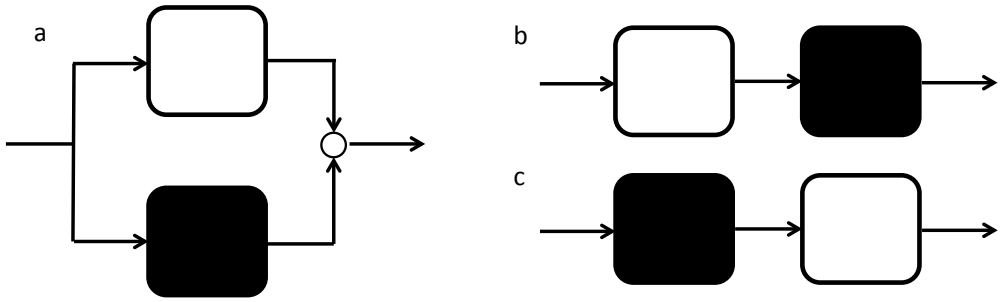

Figure 2-7. Schematic sketch of a) parallel and b) and c) serial hybrid model structures. Mechanistic and data-driven models are represented by a white and a black box respectively.

2.8.2. Application of hybrid modeling in other disciplines

The first application of hybrid modeling dates 1992 [120] as a tool for process modeling and optimization. Many other studies followed addressing the issues of hybrid model structure,

hybrid model identification and applications in the process industries. These have been recently reviewed in von Stosch et al. [119]. The most frequently addressed problem is modeling of chemical reactors (e.g. [121,122] and biochemical reactors [123,124]). Oliveira [125] proposed a general formalism for bioreactor dynamic modeling, upon which a MATLAB toolbox has been developed and further extended to systems biology problems [126]. To a lesser extent, process separation units have been studied, as for instant chromatography [127], crystallization [128] and distillation columns ([129]. While noticeable progresses have been made, many challenges remain in different fields. In a recently featured Biotechnology Journal [126], hybrid modeling has been highlighted as a high potential tool for Quality by Design (QbD) in Process Analytical Technology (PAT). In the pain management field, Clifton et al. [130] developed a promising hybrid statistical and mechanistic mathematical model for a personalized guide for mobile health intervention for chronic pain.

2.8.3. Benefits of hybrid modeling and its application to CDR

Hybrid modeling is a well-studied approach in process systems engineering [[119] which provides many benefits, by complementing the process knowledge from first-principles with the simplicity and low computational cost of the data-driven model [119,125,127]. The key idea is constraining data-driven identification by prior knowledge regarding process mechanisms. One advantage is that predictions of a data-driven model can be bound to abide physical limits, as for instance preventing concentrations of compounds to become negative or mass fractions that do not sum to unit. Moreover and foremost, constraining data-driven model by reliable prior knowledge might reduce the complexity of the identification problem thereby reducing the amount of experimental data required to identify a possibly less complex data-driven model [131].

Conceptually, these advantages can be extended in the modeling of controlled drug release using hybrid modeling methodologies. This thesis presents a first attempt to adopt hybrid modeling methodologies for the modeling of drug release and optimization of drug-carrier encapsulation. Currently different sources of knowledge about the controlled drug release phenomena are already available in the form of mechanistic and empirical equations. It is expected that a serial hybrid model (see section 2.8.1) structure comprising one knowledge-based block in addition to a data-driven model can be used to model the drug release (particularly the initial burst). In this serial structure, the data-driven model will represent the variables of the knowledge-based model for which it is difficult to derive mathematical expressions. This approach allows describing and understanding a system, even though the

underlying phenomena are unidentified at the outset, as for instance, for a new drug-carrier system. Ultimately, the hybrid model approach will be integrated in an optimization framework, for a rational design of the carrier of a specific drug, with a pre-defined burst release.

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Chapter 3 - Modeling of the burst release from PLGA micro- and nanoparticles as function of physicochemical parameters and formulation characteristics

ABSTRACT

A substantial drug release from poly(lactic-co-glycolic) acid (PLGA) micro- and nanoparticles can occur in the first hours of immersion, which is referred to as burst release. A strong burst release (when not intentional) is to be avoided as it decreases the efficacy of the treatment and could be dangerous to the host. In this work we analyze the total amount of drug released during burst and respective kinetics in relation to formulations characteristics, experimental conditions and drug molecular properties in 152 drug release experiments with 41 different drugs by partial least squares (PLS) and decision tree regression. The model created enables to quantify to which degree the physicochemical parameters control the burst release from PLGA particles. Our analysis shows that the amount of drug released during burst is mostly influenced by the formulation characteristics and the synthesis parameters, whereas the drug release kinetics is also influenced by the molecular properties of the drug. The variables that significantly influence the amount and kinetics of the burst release are discussed in detail and compared with findings from other researchers. The final regression models are shown to predict the release profile of a new drug, opening the possibility to be applied to systematically manipulate the burst release by means of designing an optimized drug delivery system.

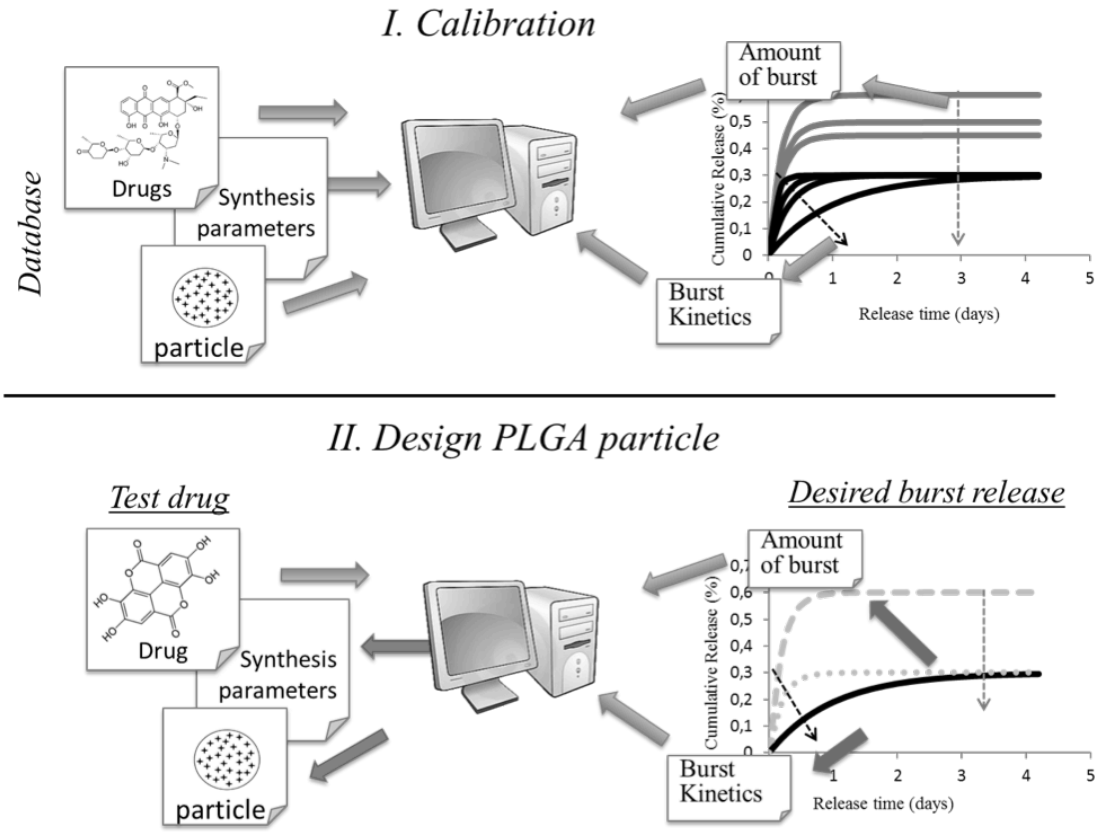

Figure 3-1. Graphical Abstract

Keywords

Controlled Drug Release, Burst release, Decision trees, Partial least squares, PLGA carriers

3.1. Introduction

A rapid release of drug can occur during the first hours of immersion of a controlled drug release system [1–7]. This burst release is due to the leakage of the drug located near the particles surface. Also a poorly entrapped drug easily diffuses out of the carrier during the initial contact with the biological fluid, giving rise to a strong burst. In a few particular cases it can be designed as a triggered burst [8], but usually it is an uncontrolled, undesired phenomenon (see e.g., review of Huang et al. [9]). An intense burst drug release often leads to a shorter total drug release time and high initial drug concentrations that can have deleterious effects on the host. Moreover, the burst release hinders predictability and reproducibility, which are prerequisites for a safe controlled drug release system. Although the burst phase is well documented, no consensus seems to exist on the underlying mechanisms, as stated by Huang et al. in a review on the mechanisms of burst release [9].

Modeling of drug release from micro- and nanoparticles based on a mechanistic or phenomenological interpretation has been addressed in many previous studies (e.g., [10–17]). However, the experimental conditions and drug properties, which have a significant impact on the controlled release profile, are only implicitly taken into account by these models. More specifically, the model parameters that characterize the drug release profile are identified from data and their values are context dependent implying that for new drugs and experimental conditions the parameters are per se unknown. The average carrier radius is typically included in such models, enabling to study the impact of the carrier size on the drug release profile. However, the carrier chemical composition (e.g., monomers ratio), which has a significant impact in the controlled release profile, is indirectly represented by model parameters and cannot be extrapolated to a different context. Thus these models are often unfit to predict the effect neither of the carrier chemical composition, nor of the experimental conditions, formulation characteristics or drug chemical properties on the controlled release profile.

Data driven approaches have also been used for controlled drug release modeling and analysis. These methods can be used to identify significant correlations between macroscopic kinetic parameters and physiochemical properties of the carrier and/or drug without a formal mechanistic interpretation. Matero et al. [18] divided the cumulative drug profile into different phases and then applied Partial Least Square regression (PLS) to each phase individually, using molecular descriptors as inputs. The final PLS models were however specific to the type of hydrophobic matrix tablet studied. Szlęk et al. and Zawbaa et al. [19–21] used the formulation characteristics and time in addition to physiochemical properties of the drug (molecular descriptors), as inputs to a number of data-driven approaches (genetic algorithms, artificial neural networks, random forests, multivariate adaptive regression

splines, etc.) for modeling the cumulative amount of drug released from Poly(lactic-co-glycolic acid) (PLGA) microparticles. While the drug release profiles were fairly well modeled, understanding how the formulation characteristics or drug properties impact on the drug release, i.e.,on the amount of drug released or the release kinetics was not straightforward due to the empirical nature of the models.

In this paper, the main objective is quantify to which degree the physicochemical parameters control the burst release from PLGA particles, enabling in a second step a rational design of the carriers for an optimized initial drug release profile. PLGA micro- and nanoparticles were chosen due to their widespread use as controlled delivery carriers [22]. PLGA particles are biodegradable and biocompatible. The fine-tuning of PLGA particles mechanical properties and consequent degradation rate is possible. Moreover, PLGA particles have been approved by the FDA in several PLGA-based drug products [23]. A thorough review on modeling of controlled release from PLGA microparticles can be found in Versypt et al. [24]. Herein, we focus uniquely on the burst phase. We use the model proposed by Corrigan et al. [25,26] to calculate the total amount of drug released during burst and respective first order kinetic constant for 152 release profiles found in literature. Subsequently, the impact of drug characteristics, carrier characteristics and synthesis parameters on the amount and kinetics of burst release are separately studied by empirical regression models. PLS and decision trees regressions were used for this purpose because both techniques can be applied to problems where the number of input variables is equal or higher than the number of independent observations. But foremost both techniques enable to robustly discriminate the relative importance of input variables from a large set of input variables [27–29].

3.2. Materials and methods

3.2.1. Data assembly: Material properties, Drug Carrier Design and Experimental Conditions

The database from Szlęk et al. [21] was curated and extended with data from literature. This resulted in a dataset comprising 152 *in vitro* controlled release experiments with 41 different active pharmaceutical ingredients (hereinafter referred to as "drug" for simplicity) that have wide therapeutic applications (Table 3-1) and a total of 74 descriptor variables (Table 3-2). The dataset comprises i) the cumulative drug release profiles over time, ii) molecular descriptors of the drug and iii) formulation characteristics of the drug-carrier synthesis. The molecular physiochemical properties of the drug are represented by 50 molecular descriptors (MDs). The ChemAxon plugin from Marvin (v5.2.1 [30]) was adopted to calculate 114 MDs, 50 of which were found unique information. These 50 MDs comprise physicochemical property predictors, and structural property descriptors such as simple elemental analysis descriptors, topological polar surface area or molecular surface area descriptors (Table 3-4). The formulation characteristics were extracted from the protocols of the drug-loaded PLGA particles preparation methods described in literature (Table 4-2). The variables of formulation characteristics comprise i) PLGA chain composition, ii) emulsifier and solvent utilized, iii) specimens concentrations during drug-carrier synthesis, iv) size of the carrier and v) the method of synthesis.

Table 3-1:Drug names and source of data utilized in this study

Drug designation	Abbreviation	Source
Aclacinomycin	ACM	[31]
Alpha-1 Antitrypsin	α1AT	[32]
Alpha-Chymotrypsin	AChT	[33]
Amoxicilin	AMX	[34]
Amphotericin B	AmB	[35]
Amyloid beta	$A\beta_{1-15}$	[36]
Bovine insulin	B-INS	[37]
Bovine serum albumin	BSA	[38–41]
Camptothecin	CPT	[42]
Cisplatin	CIS	[43]
Clonazepam	CLZ	[44,45]

Curcumin	CUR	[46]
Daunorubicin	DAU	[31]
Dexamethasone	DXM	[47]
Doxorubicin	DOX	[31]
ellagic acid	EA	[48]
Epirubicin	EPI	[31]
Estradiol	EST	[49]
Etoposide	ETO	[50]
Exenatide (synthetic exendin-4)	EXE	[51]
(5-)Fluorouracil	5-FU	[52]
Gamma-chymotrypsin	GChT	[51]
Human serum albumin	HSA	[26]
Idarubicin	IDA	[31]
Indomethacin	IND	[26]
Insulin	INS	[53]
Ketoprofen	KET	[26]
L-asparaginase	L-ASP	[54]
Lysozyme Recombinant Protein	LZM	[38]
Minocycline	MIC	[55]
Methanone	CB13	[56]
Nalmefene	NAL	[57]
Ovalbumin	OVA	[26]
Paclitaxel	PTX	[58,59]
Quercetin	QCT	[60]
Recombinant Human Epidermal Growth Factor	rhEGF	[5]
Recombinant human erythropoietin	rhEPO	[61]
Risperidone	RIS	[62]
Ropivacaine	ROP	[63]
Tumor necrosis factor receptor	OX40	[64]

Vincristine	VCR	[42]

Table 3-2: Description of variables of input for regression models

Input number	Description
1-50	Molecular descriptors of the drug Calculated using MarvinSketch [65]
51-74	Formulation characteristics
51	PLGA molecular weight
52	Lactide to glycolide ratio in PLGA
53-55	Type of emulsifier
56-58	Type of solvent
59-60	Emulsifier concentration (%)
61-62	Emulsifier molecular weight
63	Encapsulation efficiency, %
64	Initial drug loading, %
65-67	Mean particle diameter, D
66	D^2
67	$1/D^2$
68	Use of PEG, yes/no (y/n)
69	PLGA concentration
70	PLGA to PEG ratio
71	w/o/w, water-in-oil-in-oil method (y/n)
72	s/o/w, solid-in-water-in-water method (y/n)
73	s/o/o, solid-in-oil-in-oil method (y/n)
74	o/w, oil-in-water method (y/n)

3.2.2. Release profiles extraction

When the cumulative release profiles were not explicitly reported, the cumulative concentration of drug released on the immersion medium along time was extracted from the articles via the image recognition software Plotdigitizer (version 2.6.8).

3.2.3. Modeling of the drug release profile

Several mathematical models have been proposed to describe drug release, as reviewed by Siepmann et al. [66] and Arifin et al. [67] . The model by Corrigan et al. [26] was chosen because it is the only model that explicitly accounts for the burst phenomena, instead of incorporating it in the posterior phase (drug release controlled by diffusion). The first term of the Corrigan model (equation 1) describes the fractional quantity of drug released during burst over time assuming it follows first order release kinetics:

$$\boldsymbol{Q = F_{B,in} \cdot (1 - \exp(-k_b \cdot t))} \qquad \textbf{(1)}$$

where Q is the total fraction of drug released at a given time *t*, (a value between 0 and 1), $F_{B,in}$ is the fraction of drug released during the burst, k_b is a first order rate constant associated with the kinetics of the burst release. It is considered that no drug is released at the beginning of the experiment Q(t=0)=0). The end time of burst release was determined by calculating the first inflection point of the fitted curve of the drug release profile, before the lag phase. The determination of the end-point might introduce some bias as the burst phase might not yet or already been completed at the determined point. In particular this determination might have an impact on the estimated total amount of drug released during burst for each profile. However, it can be expected that for an increasing number of profiles the estimation error goes on average towards zero, wherefore the subsequent techniques should not suffer from a systematic bias. The parameter values of $F_{B,in}$ and k_b were estimated for each of the 152 drug release profiles using the Matlab function "lsqnonlin", which employs the Marquardt-Levenberg method for nonlinear least squares regression. In order to estimate the parameters confidence intervals, Monte Carlo sampling (100 repetitions) was applied on the experimental data assuming a standard error of 2.5% of the experimental value.

3.2.4. Data Pre-treatment

The database comprises 78 variables (50 molecular descriptors of the drugs, 24 formulation variables, the kinetic parameter (k_b) and the fraction of drug released during the burst ($F_{B,in}$) and the respective confidence limits) for 152 drug release profiles from PLGA carriers. These data were pre-treated as follows. The categorical variables were transformed into binary ones using a "dummy variable" approach (e.g., "use of polyvinyl alcohol? yes=1, no=0"). The distributions of the k_b and $F_{B,in}$ data were analyzed and transformations were applied (

$F_{B,in}{}^{1/4}$ and $\log_e(k_b)$) to achieve approximated normally distributed values. All data was further pretreated by removing co-linear variables and by linear auto-scaling. In the case of PLS models, two variables containing missing values were excluded from the analysis.

3.2.5. Regression Models

Pretreated data of the target output variables $F_{B,in}$ and k_b were regressed against the pretreated data of drug molecular descriptors and formulation characteristics. Firstly, the dataset comprising 152 controlled release experiments were partitioned into a training-validation subset (132 experiments) and a test subset (20 experiments). For each of the target output variables, $F_{B,in}$ and k_b , separate PLS and decision tree models were developed as described below

3.2.5.1. Partial Least Square (PLS) Models

PLS was applied to regress the parameters of the Corrigan model, $F_{B,in}$ and k_b, (outputs, Y) against the molecular descriptors and formulation characteristics (inputs, X), (equation 2),

$$\boldsymbol{Y = X \cdot \beta + \epsilon} \qquad \textbf{(2)}$$

where β represents the regression coefficient and ϵ is the residual. In essence PLS maximizes the covariance between the inputs and the outputs by decomposing and correlating the variances in a latent variable space. For a detailed description see [68]. The N-way toolbox created by Bro and Andersson [69] with default settings was used for the identification of the parameters. The training-validation set was 100 times randomly partitioned into a training (80%) and validation set (20%). The training set was used to identify the parameter values and the validation set was used to determine the optimal number of latent variables (cross-validation), i.e.,the number of latent variables for which the lowest mean squared error (MSE) is obtained. In total 100 PLS models were obtained, one for each partition. The overall output predictions were aggregated by averaging the output predictions of the 100 PLS models. Confidence limits for the predictions were obtained by computing the standard deviation of the 100 predictions.

3.2.5.2. Decision Trees

Decision trees were applied as above to regress the Corrigan model parameters, $F_{B,in}$ and k_b, (outputs, Y) against the molecular descriptors and formulation characteristics (inputs, X). Decision trees are machine-learning methods that work by repeatedly partitioning the continuous data (input) in branches and by fitting a prediction model in each partition (node) to a target value (output). The resulting model can be visualized as a decision tree, where the hierarchical importance of the inputs on the outputs becomes graphically visible. Morgan and Sonquist [70] proposed a decision trees fitting algorithm to predict a quantitative output

named Automatic Interaction Detection (AID). This algorithm performs stepwise partitions starting with a single cluster of cases and searches a candidate set of predictor variables for a way to split the cluster into two clusters. For a detailed description of this and other frequently adopted regression trees algorithms see [71]. The Matlab function “fitrtree” with default settings was used to create binary regression trees, i.e., each cluster of data arriving in a node is divided into two sub-clusters. Apart from the encapsulation efficiency and the initial drug loading, which were removed during the PLS analysis due to missing values, the same inputs and the outputs (response) were adopted. The "fitrtree” function works by partitioning the tree until a quadratic error per node drops below a predefined tolerance. The decision tree was pruned with 75-fold cross-validation using 90% of the data for training and 10% for validation, i.e.,an overly large tree was grown on the training data and subsequently the branches were removed to improve the MSE performance for the independent validation data [72]. The reduction of the size of the tree i) reduces the complexity of the regression tree, ii) avoids overfitting; and iii) increases the predictive (extrapolation) capabilities of the final model [73,74].

3.2.6. Criteria for Model Performance and Input Importance

Model performance and input importance criteria were used to assess the performance of the PLS and decision tree regressions. The mean square error (MSE) is a widely used qualitative measure to evaluate the fit of the model predictions with the experimental data. Its calculation is based on the average squared distance between the prediction and the experimental target values, i.e.:

$$\boldsymbol{MSE} = \frac{\mathbf{1}}{\boldsymbol{n}} \sum_{\boldsymbol{i=1}}^{\boldsymbol{n}} (\boldsymbol{y}_{\boldsymbol{prediction,i}} - \boldsymbol{y}_{\boldsymbol{experimental,i}})^{\mathbf{2}} \quad \textbf{(3)}$$

where n is the number of observations.

In the case of PLS regression, the relative importance of each variable on each individual response was evaluated by means of the calculation of the PLS model regression coefficient β from equation (2) and the variable importance on projection (*VIP*). The *VIP* quantity for each variable is a weighted sum of squares of the PLS weights (w), taking into account the amount of explained Y-variance in each dimension. The “greater than one” rule is generally used as a criterion for variable selection because the average of squared VIP scores is equal to 1. The VIP value for the j variable is defined as

$$\boldsymbol{VIP_j} = \sqrt{\frac{\boldsymbol{p}}{\sum_{\boldsymbol{m=1}}^{\boldsymbol{M}} \boldsymbol{SSy}} \cdot \sum_{\boldsymbol{m=1}}^{\boldsymbol{M}} \boldsymbol{w_{mj}^2} \cdot \boldsymbol{SSy}} \quad \textbf{(4)}$$

where p is the number of variables, M the optimum number of latent variables, w_{mj} the weight of the j-th variable for the m-th latent variable and *SSy* is the percentage of y explained by the m-th latent variable [75]. The PLS regression coefficients, β_j , were used to determine if a

given variable has a positive or negative impact on the response. A positive or negative value of β_j signifies that the corresponding variable has a positive (+) or negative (-) impact on the response.

3.3. Results and Discussion

3.3.1. Burst release modeling – Identification of drug release model parameters

The $F_{B,in}$ and k_b values and confidence intervals were estimated for each of the 152 drug release experiments by fitting the Corrigan model (equation 1) to the experimental drug release profiles. An illustrative example of the Corrigan model fit is shown in Figure 3-2. The resulting parameter values for the 152 drug release profiles are shown in Figure 3-3. It can be seen that the values of $F_{B,in}$ vary between 0.1 and 0.95, while the values of k_b vary between 0.05 and 50 days^{-1}. Some of the confidence intervals are very broad relatively to the parameter values. Since $F_{B,in}$ and k_b are the target outputs for PLS and decision trees regression, the experiments with wider confidence intervals (i.e., larger than 15% of the parameter value) were excluded from the calibration step. These experiments were rather used for model testing (testing sets - red lines). It can be seen that the confidence intervals are slightly tighter for $F_{B,in}$ than for k_b in the case of the training data set. For simplicity, the identified $F_{B,in}$ and k_b parameter values will be referred to as "experimental values" in the remaining of the text.

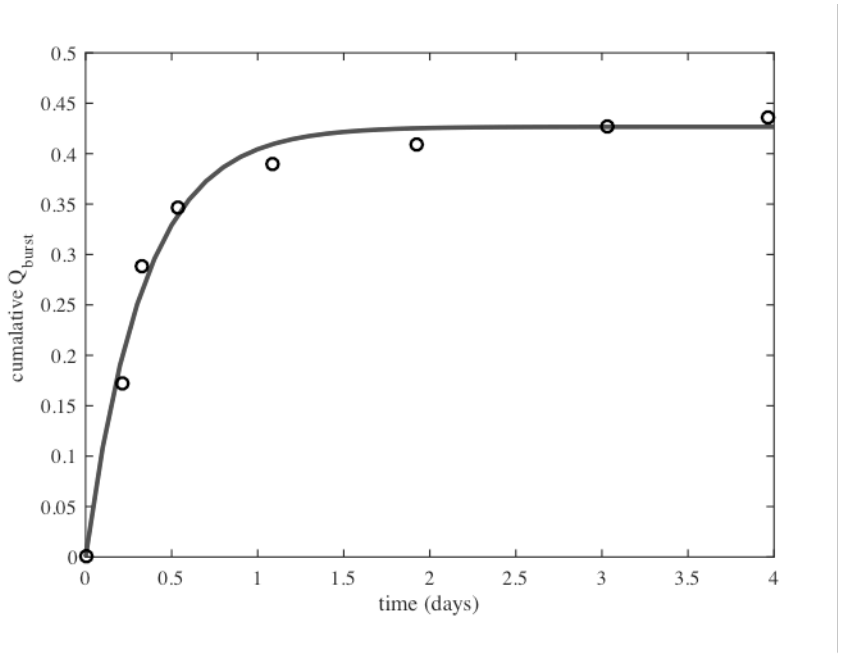

Figure 3-2: Example of cumulative release of ellagic acid from PLGA nanoparticles during burst [47]. Black circles: experimental data, continuous line, fit with Corrigan model (equation 1).

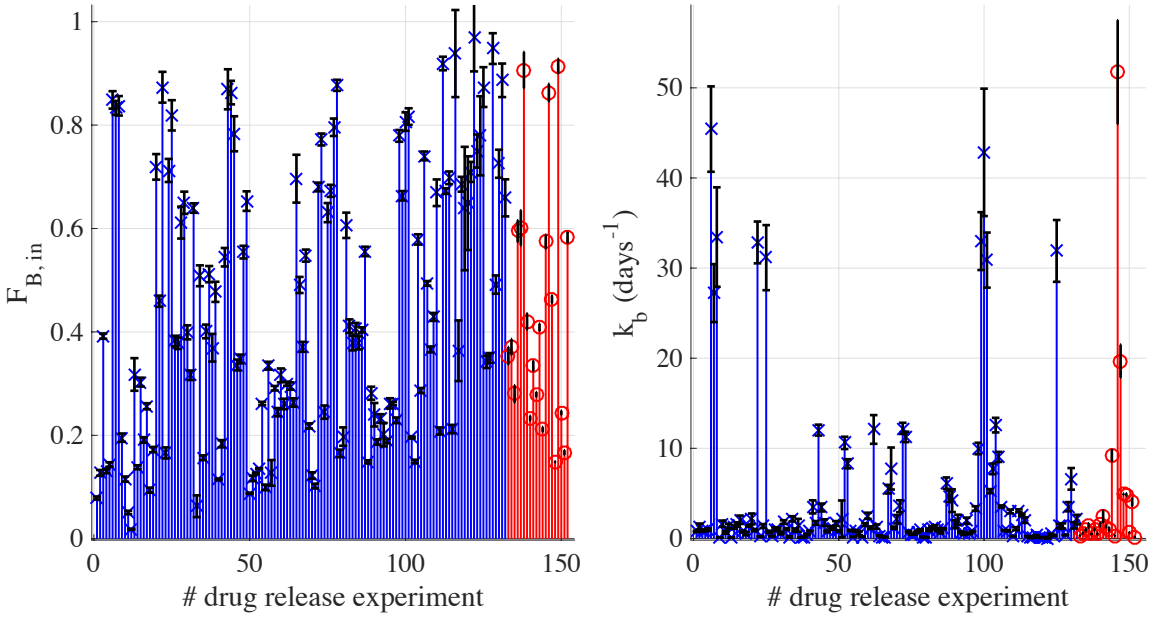

Figure 3-3: Values and standard deviations of $F_{B,in}$ (left) and k_b (right) for different experiments. Blue crosses: training set, red circles: test set, black vertical lines: standard deviation.

3.3.2. Regression Model Quality

3.3.2.1. Partial Least Square Analysis

An individual bootstrap aggregated PLS model was developed for each response variable ($F_{B,in}$ and k_b) as described above. The obtained MSEs for the $F_{B,in}$ model are 0.49 and 0.53 for the training and independent test sets, respectively. In the case of k_b , the MSEs are 0.44 an d 0.73, respectively. The predicted $F_{B,in}$ and k_b against their experimental values are shown in Figure 3-4. The $F_{B,in}$ model residuals are lower than those of the k_b model. A few outliers can be spotted, particularly for high values of $F_{B,in}$ and k_b. One possible justification for the higher residuals could be some inherent process nonlinearities that cannot be captured by PLS regression.

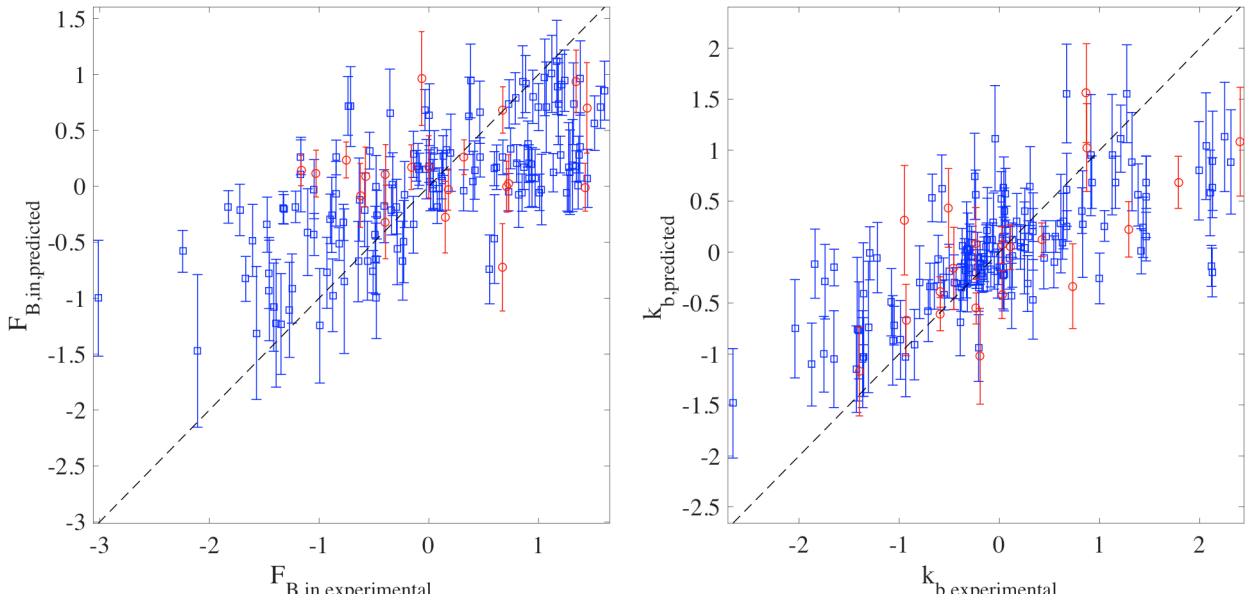

Figure 3-4: Predicted versus experimental values for $F_{B,in}$ (left) and k_b (right) obtained with PLS regression. Blue squares: training set, red circles: test set. Standard deviation of predictions obtained with cross-validation represented by vertical bars.

3.3.2.2. Decision Tree Analysis

Individual bootstrap aggregated decision tree models were developed for $F_{B,in}$ and k_b. In the case of $F_{B,in}$, the pruning technique resulted in a tree with 15 splits. The MSE obtained for the training and test sets were 0.15 and 0.18, respectively. For k_b, the best performing regression tree had a pruning of 15 splits. The MSE for training and test sets were 0.22 and 0.11, respectively. In Figure 3-5 the predicted $F_{B,in}$ and k_b are plotted against the respective experimental values. It can be seen that modeling errors are significantly lower for decision tree regression for both $F_{B,in}$ and k_b when compared to PLS regressions. Some outliers are still observed, predominantly for the test set which contains experiments with wider confidence intervals.

Overall the fit of the models is good, wherefore the analysis of the impact of the material properties and experimental conditions on the response variables is enabled.

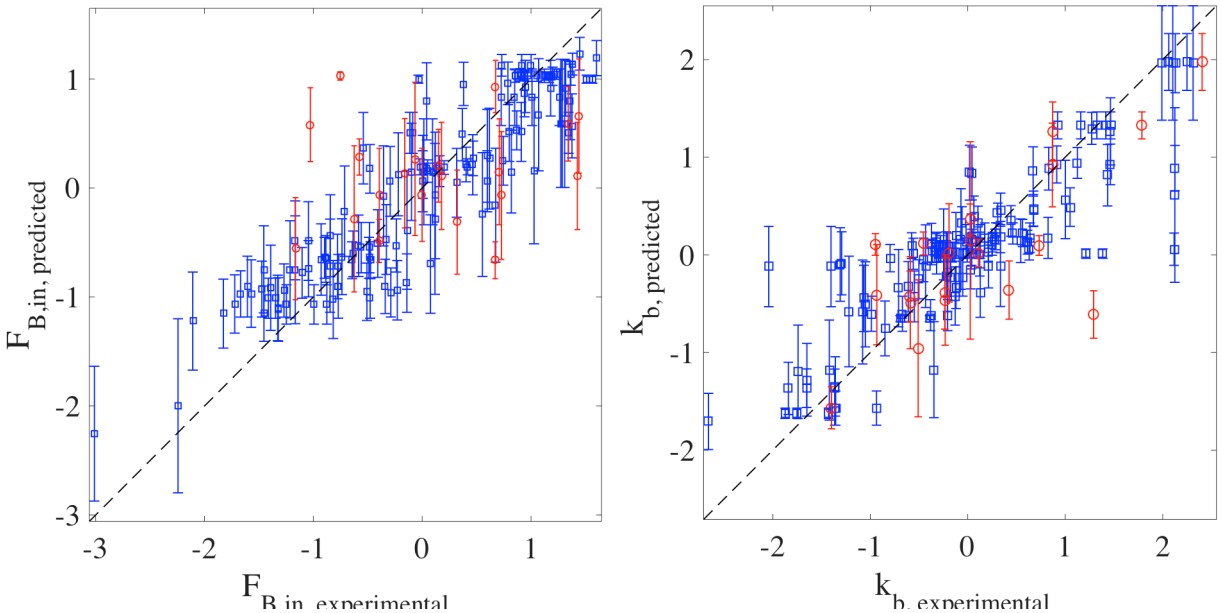

Figure 3-5: Comparison of predicted and experimental values for decision tree regression for $F_{B,in}$ (left) and k_b (right). Blue squares: training set, red circles: test set. Standard deviation of predictions obtained with cross-validation represented by vertical bars.

3.3.3. Analysis of the impact Factors

3.3.3.1. Partial Least Square

In Figure 3-7 the VIP_j values for each input variable, j, are shown. If the corresponding β_j value is positive, the bar is blue, while red if negative. By inspection of Figure 3-7 the relative impact of each variable on the different responses can be analyzed. It seems that the molecular descriptors: fused aliphatic ring count (-), fused ring count (-), largest ring system size (-) and minimal projection radius (-); as well as several formulation characteristics: PLGA molecular weight (-), ratio of lactic/glycolic acids in the PLGA chain (+), poloxamer 188 (+), TPGS (-), SDS (-) or DCM (+) during the nanoparticle synthesis, PVA concentration in the inner phase (-), molecular weight of: PVA (+) and other emulsifiers, nanoparticle size (-), D2 (+), PEG copolymer use (-), PLGA/PEG ratio (-) and synthesis type (w/o/w (-) and s/o/w (+)); have the greatest impact on the amount of drug released during burst ($F_{B,in}$).

In the 100 derived PLS models the following factors showed both, a positive and negative impact on $F_{B,in}$ wherefore the direction of their impact is not conclusive and not further analysed: fused rig count, L/G ratio, PEG use, polax 188, D2, use of PEG and W/O/W.

In case of the drug release dynamics, k_b, the molecular descriptors with greatest impact seem to be the fused aliphatic ring count (+), the Dreiding energy (-), largest ring size (-) and the drug mass (-). In terms of formulation characteristics the following seem to have a significant

impact on k_b: the ratio of lactic/glycolic acids in the PLGA chain (-), use of DCM (+), nanoparticle size (-), nanoparticle size squared (+), incorporation of the co-polymer PEG (+), PLGA concentration (-), and the synthesis methods s/o/w (+), s/o/o (-) and o/w (-). For both $F_{B,in}$ and k_b, more variables of the formulation characteristic are selected than drug molecular descriptors. Such findings have the advantage of being characteristics able to be manipulated, for instance in the design of an optimal drug carrier. In the 100 derived PLS models the following factors showed both, a positive and negative impact on k_b wherefore the direction of their impact is not conclusive and not further analysed: the Dreiding energy, largest ring size, s/o/o and o/w.

3.3.3.2. Decision Trees

In Figure 3-6 the impact of input variables on the response variables is shown for each of the 75 tree models. The frequency of the variables and their color gradient across the tree models provides an indication of the importance of the variable for modeling the response variables. Variables that appeared in more than 60% of the tree models were analyzed further. Interestingly, this subset of variables is consistent with the VIP selection based on the PLS regression models as shown in Figure 3-7.

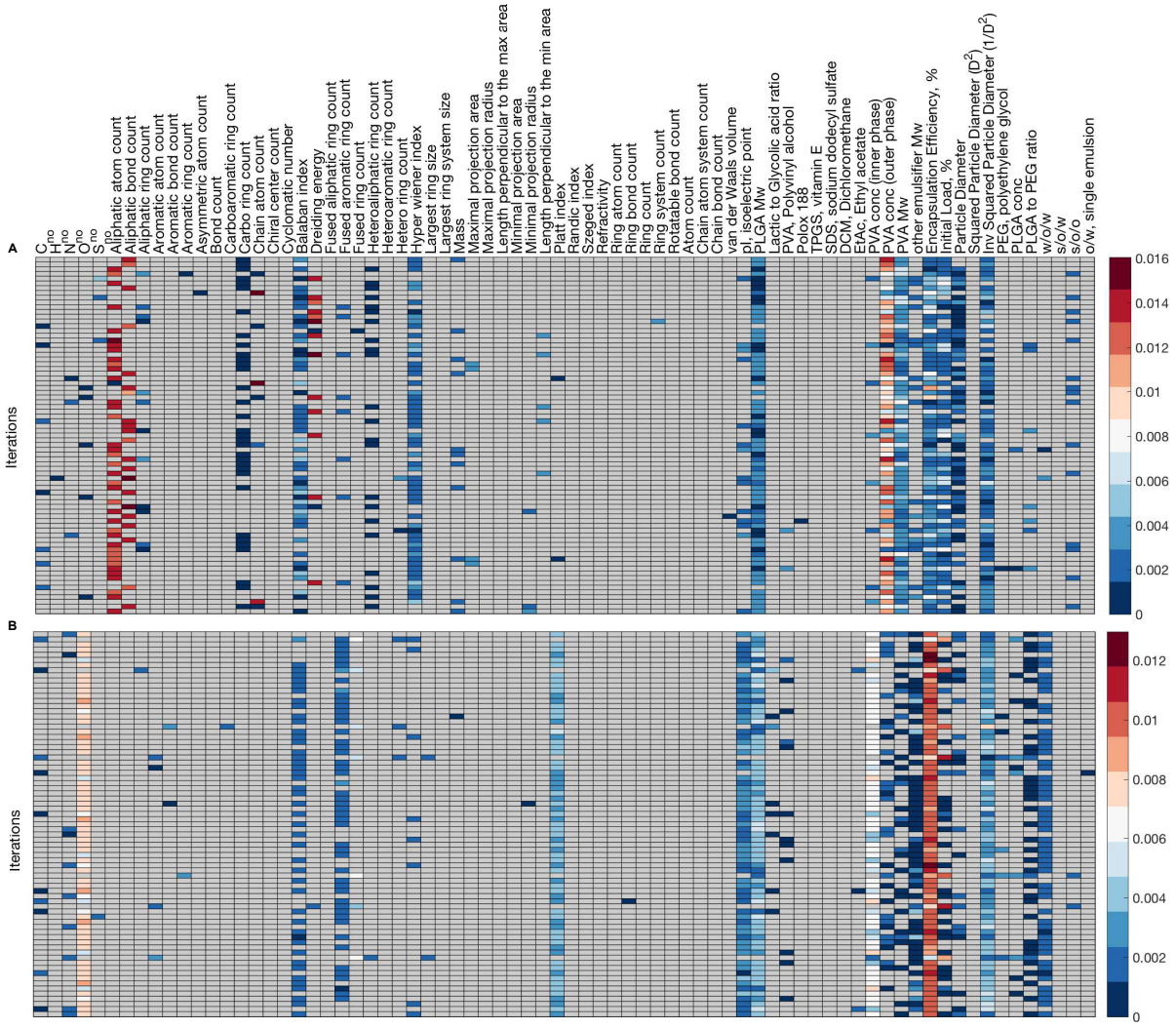

Figure 3-6: Importance magnitude of inputs for modeling of (a) $F_{B,in}$ and (b) k_b using decision tree regressions, across 75 fold cross-validation iterations.

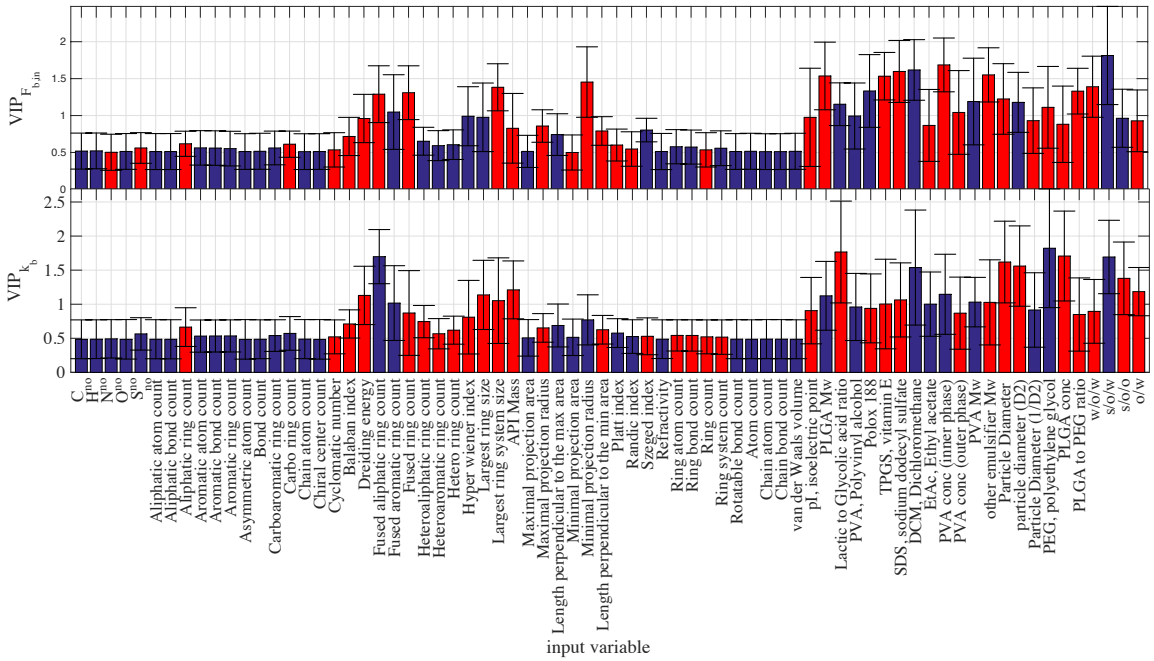

Figure 3-7: VIP values of PLS regressions inputs for $F_{B,in}$ (top) and k_b (bottom). Blue bars correspond to positive β_j values, while for negative β_j the bars are red. A positive or negative value of β_j signifies that the corresponding variable has a positive (+) or negative (-) impact on the response.

An additional tree graph has been created using all training and validation data with the optimal prune structure (Figure 3-8 and Figure 3-9). The order in which the decisions (splits) are made is shown in square brackets at each node of the Figure 3-8 and Figure 3-9. The order of splits is an indication of the importance of a given decision on the prediction of the responses $F_{B,in}$ and k_b.

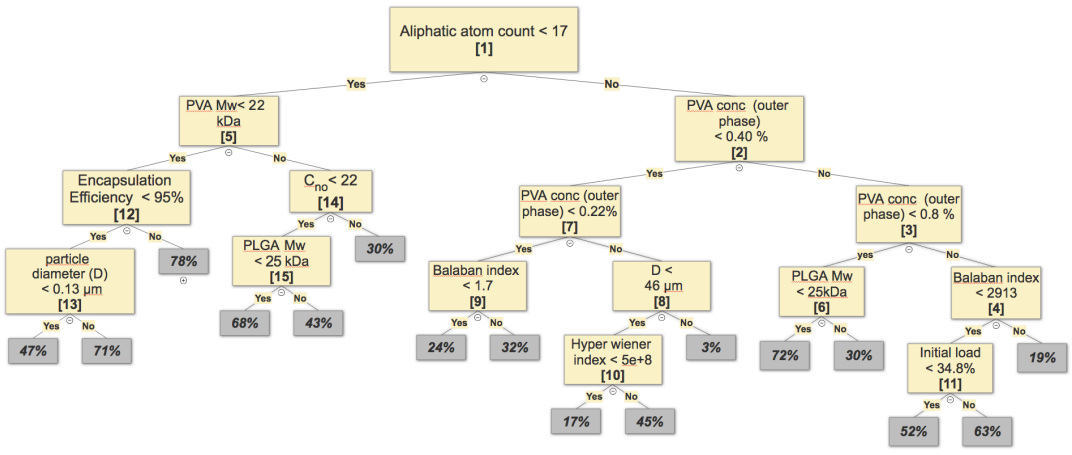

Figure 3-8: Regression tree graph for $F_{B,in}$ obtained with the training set and a maximum number of 15 splits. $F_{B,in}$ values are in percentage (%) and italic. Legend: C_{no}=number of carbon atoms in the drug.

It seems that the formulation characteristics input variables have a greater impact on $F_{B,in}$ than the molecular descriptors throughout the entire cross-validation, namely the PLGA molecular weight, the molecular weight of PVA, the PVA concentration in the outer phase, as well as the size of the particles (Figure 3-6a). Also the encapsulation efficiency and the initial drug load was found to impact on $F_{B,in}$. The encapsulation efficiency and initial drug loading were not included in the PLS analysis since a number of values in this variables were missing, which would have to be imputed for PLS analysis, but not for decision trees. The molecular descriptors with a significant impact on the target variables were the aliphatic atom count, the hyper wiener index and the Balaban index.

For k_b (Figure 3-6b) similar conclusions as for $F_{B,in}$ can be taken. The formulation characteristics have in general a greater impact on the response k_b than the molecular descriptors. The formulation characteristics with a higher impact on k_b were the PLGA molecular weight, PVA concentration on inner phase, the molecular weight of surfactants (other than PVA), the encapsulation efficiency, the particles size ($1/D^2$), the PLGA/PEG ratio and the w/o/w synthesis method. The molecular descriptors with noticeable impact on the k_b are the number of oxygen atoms in the drug, the fused aromatic ring count, the Platt index and the isoelectric point (pI).

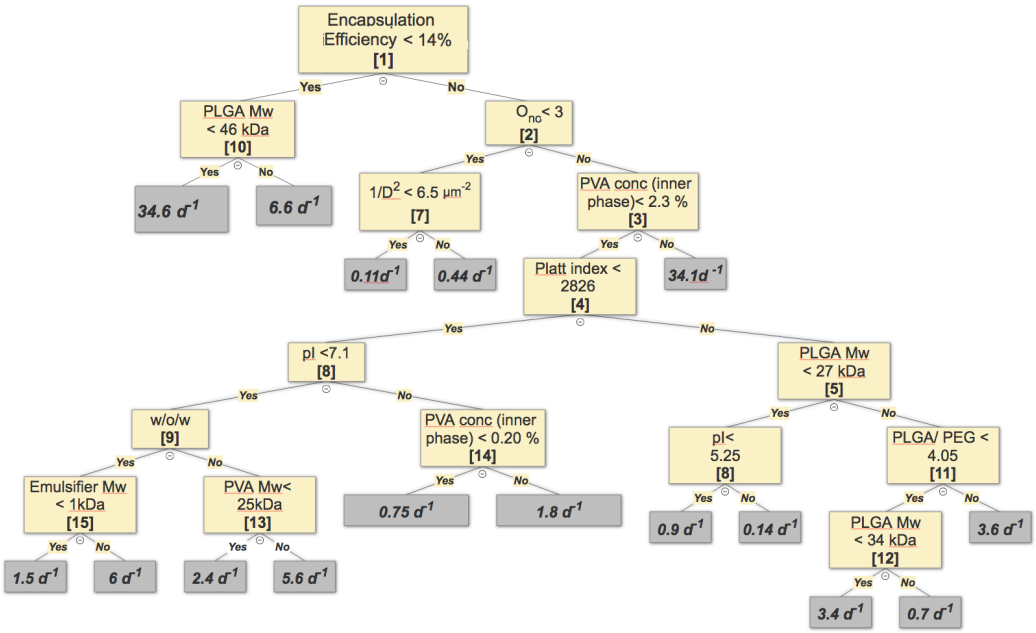

Figure 3-9: Regression tree graph for k_b obtained with the training set and a maximum number of 15 splits. k_b values are in days^{-1} and italic. Legend: O_{no}=number of oxygen atoms in the drug.

3.3.4. Discussion of the impact factors

3.3.4.1. Factors influencing $F_{B,in}$

PLGA polymer molecular weight: The size of the polymer chain was found to be a variable of importance in both decision tree and PLS regressions. The PLS model indicates that $F_{B,in}$ decreases for increasing PLGA chains (negative sign). This result is in agreement with several authors who reported that particles produced with PLGA with lower molecular weight exhibit a more accentuated burst [38,43,49,76–79]. Mehta et al. [79] suggested that the faster solidification rate of polymers with higher molecular weight leads to high encapsulation efficacy values (as observed by Jeyanthi et al. [80]). This in turn implies that particles synthesized with low molecular weight PLGA will have higher microporosity such that more inner channels may facilitate the drug located near the particles surface to escape.

PVA molecular weight: According to both the PLS and decision tree analysis the PVA molecular weight was found to have a significant impact on the amount of burst. PLS results indicate that an increase of molecular weight of the surfactant PVA used during particle synthesis increases the amount of drug released during burst. An increase of PVA molecular weight results in a decrease of its solubility in water [81]. Though the influence of the PVA concentration during particle manufacturing on the drug release has been widely studied (section below), to the best of our knowledge, the impact of variations of the PVA molecular weight on the drug release was not yet studied. Thus, it is not clear whether and what mechanisms are behind the impact of the molecular weight of this surfactant on the amount of burst. Given the outcome of the regression models, this factor should be studied in the future.

PVA concentration inner phase: The PLS results indicate that an increase of the PVA concentration in the aqueous inner phase slightly decreases the amount of drug released during burst. Such finding is in agreement with [82,83], who described that increasing amount of PVA (until a threshold of 1w/v %) in the internal water phase increases the stability of internal phase, resulting in more uniform drug distribution, hence less drug is released initially. Also microparticles prepared with low PVA concentrations have bigger inner pores, which enhances the amount of drug released upon immersion. Moreover PVA concentration in the aqueous inner phase is a key factor to influence the microparticles' size. According to [82–84], increase of PVA concentration until a threshold of 2% slightly increases the size of the microparticles and also increases the drug encapsulation efficiency; both characteristics are associated with a less intense burst release.

PVA concentration outer phase: Results from decision tree indicate that the PVA concentration in the external phase is an important factor for predicting $F_{B,in}$. Yang et al. [83] described that when PVA is used in the outer phase, it protects the emulsion droplets against

coalescence, resulting in smaller particles. However, their results did not agree with the empirical rule that "drug release is inversely proportional to the size of the microspheres" [85]. Yang et al. provided an explanation for the decrease in burst release with increasing PVA concentrations despite achieving lower particles sizes. Increasing the PVA concentration leads to an increase of the viscosity of the external phase, hindering the amount of drug available to be released. The same behavior was observed by Yallapu et al. [46] who attributed a stabilizer characteristic to the high concentrations of PVA resulting in a decrease of the burst release.

Use of TPGS (vitamin E) as surfactant: According to the PLS results the use of TPGS as a surfactant has a negative impact on $F_{B,in}$. Mu et al. [86] observed a drop in the amount of drug released during burst when TPGS was used as a surfactant instead of PVA. The particles fabricated with TPGS as surfactant had a larger size as compared to PVA. However, when TPGS and PVA were used together, the amount of drug release from the nanoparticles was higher than with PVA or TPGS alone. Particles synthesized with both surfactants were smaller than those synthesized with PVA or TPGS alone, which is the reason for the increased amount of drug released during burst according to Mu et al. [86].

Use of SDS as surfactant: The use of SDS as an anionic surfactant has a negative impact on $F_{B,in}$ as can be observed in the PLS analysis. This finding is in agreement with [87], who witnessed a decrease of the initial burst when SDS was used instead of PVA under the same experimental conditions. Xu et al. hypothesized that the particles manufactured with SDS were more likely to coalesce and form larger particles, known to have lower burst release amounts due to their high polymer surface/drug ratio.

Use of DCM as solvent: The PLS analysis indicates that the use of DCM as a solvent has a positive impact on $F_{B,in}$, which is in accordance with the finding of Yang et al. [78]. Yang et al. pointed out that this finding was not expected since microparticles produced utilizing DCM have a much smoother surface with absence of pores, such not expected to exhibit high amount of burst, as when compared with the ones utilizing acetone which were very porous and irregular. Thus, this effect should be studied in more detail.

Surfactant Mw (other than PVA): The surfactant molecular weight was found to be an important variable for the amount of drug released according to PLS analysis. It was observed that the substitution (or joint usage) of PVA with other surfactants (e.g., Poloxamer 188, vitamin E TPGS, Tween 80) might impact on the amount of burst release. It was found to increase the encapsulation efficiency of the particle [86] and hinder the hydration of the carrier by means of "masking" the surface of the particle [84].

Size of particle (D): Both decision tree and PLS analysis indicate that the size of the carrier impacts on the amount of drug released during burst. According to the PLS results, smaller particles exhibit a higher amount of released drug. This finding is in agreement with several authors [76,82] who observed an inverse correlation of the particle size to the amount of burst. The size of particles was not flagged in the decision tree analysis.

Encapsulation efficiency (EE): According to the decision tree analysis, the EE has a significant impact on $F_{B,in}$. A review article by Yeo et al. [88] suggested that any efforts to control the initial burst are hand in hand with those to increase EE [79]. Namely the amount of initial burst depends "on the ability of the polymer matrix to properly encapsulate the drug, thereby making it unavailable for immediate diffusion" [88]. However, the EE cannot be tuned independently from several other formulation parameters. For instance, it was shown that the EE decreases as drug loading increased [89]. Also Su et al. [76] observed a positive correlation between EE and the molecular weight of PLGA, the polymer concentration on the oil phase and with the size of particles.

Initial drug loading: The decision tree results indicate that the drug loading has a significant influence on $F_{B,in}$. Several authors have reported a strong influence of the initial drug loading with the intensity of the initial burst release, though with opposite impacts. Some have observed an increase in the amount of drug released with an increase of initial drug loading for hydrophilic drugs in microparticles [50,76]. A possible explanation is that during the particle synthesis, high initial drug loadings lead to large amounts of drug to adhere to the surface of the particle [6], which is immediately released upon hydration [90]. However, other researchers observed, for a hydrophobic drug in nanoparticles, that the release rate decreased with an increased drug loading [91,92]. Mu and Feng [91] suggested that an increase in the drug loading leads to more compact nanoparticles, inhibiting the water penetration into the polymeric matrix leading to less drug being available for the release. Conclusions on the impact of initial drug loading on the amount of burst release should not be drawn stand-alone, hence the importance of a combined analysis of the impact factors such as decision tree analysis.

PLGA/PEG ratio: The PLS analysis suggests that lower PLGA/PEG ratios lead to higher $F_{B,in}$. The addition of hydrophilic PEG to the PLGA polymer augments the hydration of the nanoparticle and its porosity leading to a higher amount of drug release during the initial burst. Such findings were observed by [43,77,93–95] observed a correlation on the increase in the proportion of PEG in the copolymer chains to the amount of degradation of the PLGA-PEG nanoparticles, hence having a more intense burst release.

S/O/W fabrication method: Which micro- nanoparticle synthesis method is chosen depends on the drug (e.g., hydrophilic drugs) and the desired particle size (i.e.,S/O/W is typically chosen to obtain smaller particles, range of 100nm [96]) and hence the fabrication method cannot be chosen completely freely [96,97]. In PLS analysis, the S/O/W is identified to have a significant impact on $F_{B,in}$. The positive sign in the PLS analysis may be associated with the fact that the modified s/o/w fabrication method allows for the synthesis of small nanoparticles typically associated with higher burst, supporting the findings of [98,99] who observed that S/O/W experienced more intense burst releases than the ones from W/O/W. However, this finding is in disagreement with the argument of [87] who state that "the s/o/w method can improve the stability of the encapsulated agents, increase the entrapment efficiency, and produce a less pronounced burst effect".

Aliphatic atom count and Fused aliphatic ring count: The number of atoms in aliphatic groups was found to have a significant impact on $F_{B,in}$ after decision tree analysis. Also, the PLS model suggest that the fused aliphatic ring count as a strong negative impact on $F_{B,in}$. There is a direct correlation between the increase in the "amount" of aliphatic parts in a drug and the drug lipophilicity, i.e., the more aliphatic the drug, the less soluble it is in aqueous media. The release of very aliphatic (hydrophobic) drugs requires large medium volumes, such that the concentration gradient is high enough to allow drug transport from the inside of the particle to the medium. For low medium volumes the thermodynamic balance is reached once a low amount of drug is released. Also it was observed by Ramen et al. [100] that highly hydrophobic drugs tend to have a non-uniform distribution within the carrier, being mainly located in the center of the particle. Such results in hydrophobic drugs having a slower initial release from PLGA particles due to lower amount of drug near the particle surface, readily available for diffusion. In addition, though not linearly, this MD is related to the size of the drug molecule and affects inversely its solubility in water. Therefore, it may be inversely correlated with the amount of burst release as supported by findings of Su et al. [76] and Mao et al. [82] who observed an increase on the burst intensity with the decrease of the drug mass.

Hyper wiener and Balaban indexes: Both indexes describe the topology of the drug molecule and they were found to have an impact on $F_{B,in}$ on the decision tree analysis. They describe "distances" between atoms on a drug molecule. The hyper Wiener index describes the sum of the shortest distances between non-hydrogen pairs of atoms. For molecules with identical number of atoms, the hyper wiener index is greater for more linear structures (e.g., alkanes) and lower for molecular structures with many branches and cycles [101]. The Balaban index describes the sum of distances between non-hydrogen atoms normalized by a factor, which depends on the mass of the molecule and the number of rings in the molecular structure. Sabljic et al. [101] stated that the Balaban index increases with the size of the

molecule, but, opposite to the hyper wiener index, the Balaban index increases with the degree of unsaturation and degree of branching of the molecule. Both MDs are a measure of the branching of a molecule [101]. There is no literature information on the impact of these topological MDs on the drug release to the best of the authors' knowledge. For more on these molecular descriptors see [30].

Largest ring system size and Minimal projection radius: The PLS model suggests that both MDs impact negatively on the amount of drug released. The former describes the size of the largest ring system (number of rings) in the molecule (0 when acyclic); the later refers to the projection radius of the conformer, which is based on the van der Waals radius (in Å) [65]. Though not linearly, both MDs are somehow related to the size of the drug molecule, hence an increase of their value could be inversely correlated with the amount of burst release as supported by findings of [9,102] who observed an increase on the burst intensity for a decrease of the drug mass. The minimal projection radius was also found to impact on the drug dissolution from PLGA microspheres by[19].

3.3.4.2. Variables influencing k_b

PLGA polymer molecular weight: According to the decision tree results the PLGA molecular weight has an impact on the kinetics of burst. Makadia et al. [103] stated that PLGA chains with low molecular weight show an enhanced water permeation resulting in a faster drug diffusion , which accelerates the degradation of PLGA resulting in a faster release of drug. Cohen et al. [104]observed that for large drugs (e.g., protein FITC-BSA) the burst release from PLGA carriers increases as the molecular weight of PLGA decreases. At high PLGA molecular weight, the burst release is negligible due to the relative size of the protein and polymer pores resulting in a slow mobility. Thus, when PLGA molecular weight is lower, the proteins are more movable, wherefore they are transported faster and exhibit an enhanced k_b. This characteristic is more prominent for large drug molecules [105].

Lactic to glycolic acid ratio (L/G) in PLGA polymer chain: The PLS analysis suggests that the L/G ratio in PLGA has a negative impact on k_b. The variation of the monomers ratio is known to change the degradation rate of a copolymer [106]. It was shown by Cui et al. [107] that an increase of the ratio of lactic acid over glycolic acid would delay the degradation of the polymer chain delaying as well the release of the entrapped drug. The hydrophilic nature of glycolic acid is expected to give the PLGA particle an accelerated degradation rate [108]. However, Cui et al. states that the degradation of the particle and consequent drug release is more accentuated after a few days of release and not as pronounced in the initial phase.

Size of nanoparticle, D: The particle size seems to have an impact on the burst kinetics as indicated by the results of both PLS and DT. The PLS model suggests that the particle size has a negative impact on k_b, i.e., smaller particles have a higher k_b. Such finding is in agreement with [76,82,106] who stated that the larger surface to volume ratio of smaller particles increases the diffusion of drug released to the medium and the drug encapsulated in smaller particles has shorter pathways to diffuse until the surface, hence having a faster release.

Size of the carrier ($1/D^2$): The inverse of the squared diameter of the particle was found to have an impact on k_b by the decision trees analysis. The incorporation of this variable as well as D^2 was part of a strategy to incorporate a surface-area related measure in the analysis. The conclusion that an increase in $1/D^2$ correlates with a faster burst is in agreement with several researchers [76,82,106] who stated that the larger surface to volume ratio of smaller particles increases the diffusion of drug released to the medium and that the drug encapsulated in smaller particles has shorter pathways to diffuse until the surface, hence having a faster release [106].

Size of nanoparticle, D^2: The squared size of the particle was found to have a positive impact on k_b in PLS analysis. The conclusion that an increase in R^2 correlates with a faster burst is counter-intuitive, as the discussion of the size of the particle (above) reveals that an increase in the size of the particle (D) leads to a slower burst release.

PLGA concentration: According to the PLS results the PLGA concentration during synthesis is an important variable for the prediction of k_b and has associated a negative impact on the burst kinetics. Such as also reported by Mao et al. [82], that the intensity of burst release drops significantly with increases of PLGA concentration in the organic phase, while all other formulation characteristics are constant. Likewise it was observed that incremental increases in the PLGA concentration produce larger particles, known for their reduced burst release [82]. Sah et al. [109] and Sharma et al. [84] observed that at low PLGA concentration in the solvent during synthesis, the amount of released drug was higher. Sah et al. hypothesized that at low polymer-suspending phase ratios, the resulting microparticles have a polymer structure with large numbers of inner porous and channels. Huang and Brazel [9] suggested that this low compact characteristic arising from low PLGA concentration in the solvent phase could result in faster initial drug release by i) enhanced transport of drug towards the surface of microparticles during the drying process and ii) the formation of larger pores, allowing for a faster initial release rate.

PVA concentration in the inner phase: The decision tree results indicate that the PVA concentration in the aqueous inner phase impacts the kinetics of burst. This result is supported

by [82,83], who observed that a higher concentration of PVA (until a threshold of 1% w/v) increases the stability of the internal phase, resulting in more uniform drug distribution concomitantly with slower initial drug release. Also particles prepared with low PVA concentrations have bigger inner pores thereby accelerating the drug release upon immersion. Moreover, the PVA concentration in the aqueous inner phase is a key factor to influence the particles' size. According to several researchers [82–84], increasing the PVA concentration until a threshold of 2%, slightly increases the size of the particles and also increases the drug encapsulation efficiency, both contributing to decrease the burst release.

Surfactant molecular weight (other than PVA): The surfactant molecular weight was found to be an important variable for the burst kinetics by the decision trees analysis. Though PVA is the surfactant of choice for a wide number of micro- and nanoparticle formulations, other surfactants (e.g., Poloxamer 188, vitamin E TPGS, sodium cholate and Tween 80) were studied either alone or combined with PVA. These surfactants were found to impact on the release, by means of e.g., increasing the encapsulation efficiency of the particle and by changing its hydrophobicity and associated degradation rate [84,86].

Use of DCM as solvent: The PLS analysis indicates that the use of DCM as a solvent has a positive impact on k_b. As mentioned in the section above, microparticles produced utilizing DCM have a smoother surface without pores, as when compared with the ones utilizing acetone which were very porous and irregular [78]. It would be expected that the use of DCM would have a negative impact on the kinetics of burst. The impact of this factor requires further study.

Encapsulation efficiency (EE): The decision tree analysis indicates that EE influences the burst kinetics. The EE cannot be independently changed without changing other factors, namely the synthesis method, the drug hydrophobicity or the surfactants. EE was shown to decrease with increasing drug loading [84]. Also the EE was reported to augment with i) the increase of PLGA molecular weight, ii) the increase of PLGA concentration in the oil phase, and iii) the increase of the carriers size [75].

Use of copolymer PEG: The PLS analysis indicates that the use of copolymer PEG has a positive impact on k_b. A significant faster release of drug from PLGA-PEG particles compared with that from PLGA samples was observed by a number of researchers [77,93–95]. Peracchia et al. [110] observed that the degradation of PEGylated particles was much faster than its PEG free counterparts; the coupling of hydrophilic PEG enhances the hydration of the polymer chain and subsequent degradation of the nanoparticle structure, leading to accelerated burst release kinetics (up to a 15-fold increase) [111]. Also, an augmented porosity of the PLGA–PEG particles versus their PLGA counterparts allows for a larger drug

entrapment and a facilitated subsequent drug release [112]. However, in *in vivo* studies contradictory results were obtained for additions of PEG to PLGA chains. For instance, it was observed by [113,114] that the carriers with PEG have a slower burst release when compared with the PEG-free particles. However, in this study we study *in vitro* experiments.

It is also important to stress that only experiments with PEG coupled to the PLGA chain were investigated, excluding CDR experiments where the PEG was associated with the drug (PEGylation of the protein). PEGylation should not be confused with incorporating the copolymer PEG into PLGA.

PLGA/PEG ratio: The decision tree analysis shows that PLGA/PEG ratio impacts k_b. Likewise, PLS analysis suggests that lower PLGA/PEG ratios lead to a faster burst. These results are in agreement with [43,77,93–95]. Avgoustakis et al. [43] observed a correlation between the increase in the proportion of PEG in the copolymer chains and the amount of degradation of the PLGA-PEG particles. Hence particles with higher PEG content have a steepest burst release. Peracchia et al.and Avgoustakis et al. [43,110] observed that the degradation of PLGA-PEG particles was much faster than their PEG free counterparts. The coupling of hydrophilic PEG enhances the hydration of the polymer chain and subsequent degradation of the carrier structure, leading to faster burst release kinetics (up to a 15-fold increase) [111]. Also, higher porosity of the PLGA–PEG particles versus their PLGA counterparts allows for the entrapment of a larger drug and facilitated subsequent drug release [112]. However, *in vivo* studies contradictory results were obtained. For instance, it was observed by Ito et al. [113] and Peracchia et al. [114] that the carriers with PEG have a slower burst release when compared with the PEG-free particles. It should be noted that in the present study only *in vitro* experiments are analyzed. It is also noteworthy to stress that in our study only experiments with PEG coupled to the PLGA chain were investigated. Thus controlled release experiments where the PEG is associated with the drug (protein PEGylation) are not part of our analysis.

Synthesis method: As stated above, the choice of synthesis method is highly dependent on the drug and the desired carrier characteristics. Still both PLS and DT analysis returned the S/O/W as important factor for the burst kinetics. The positive sign in the PLS analysis for S/O/W may be associated with the fact that the modified s/o/w fabrication method allows for the synthesis of small nanoparticles typically associated with higher burst [98,99]. W/O/W, an emulsifier solvent-evaporation technique was observed to produce particles with high number of pores and fissures, which Park et al. [115] and Huang et al. [9] point out as being a reason of the fast burst released observed from particles produced by W/O/W.

Drug mass: The PLS model suggest that the burst release in nanoparticles loaded with smaller drugs is faster. This agrees with expected result that smaller solutes have a faster transport than larger molecules (e.g., proteins) from the center of the particle through the internal channels and pores [9,102].

Isoelectric point (pI): The pH at which the net charge of a molecule is neutral is called the isoelectric point, or pI. The decision tree analysis indicates that pI is an important factor for the k_b prediction. The pI value can affect the solubility of a molecule at a given pH. Molecules have minimum solubility (increased hydrophobicity) in aqueous solutions when the pH equals their pI, which can result in the precipitation of the molecule. The pI along with the media pH will determine the drug charge and consequently the solubility of the drug in an aqueous medium[116]. Hence, a slower burst release should be expected for an increase of the drug's pI, due to an increase in the drug's hydrophobicity.

Platt index: This index was identified to be important for the prediction of the k_b by the decision tree analysis. The Platt index is a path-based topological molecular descriptor, which is equal to the sum of degrees of bonding in a drug molecule [30,117]. For molecules with similar masses, the one with more unsaturated atoms would have a higher Platt index [101]. In this way, the Platt index can be seen as a measure of unsaturation of a molecule. The exact impact mechanisms on the drug release are not known to the best of our knowledge.

Oxygen number of atoms and Fused aromatic ring count and fused aliphatic ring count: The decision tree and PLS model suggests that these MDs have a significant impact for the prediction of k_b. Though not linearly, these MDs are related to the size of the drug molecule, hence an increase in their value could be inversely correlated with the an increase of the kinetics of burst release in agreement with findings by Su et al. [76] and Mao et al. [82] who observed a faster burst for lower drug mass. Smaller solutes are transported faster than larger molecules (e.g., proteins) from the center of the particle through the internal channels and pores due to a higher diffusion coefficient [9,118]. However, molecules with highly electronegative atoms (O, N, F) can form hydrogen bonds with water, thus having an enhanced aqueous solubility. Siegel et al. [119] observed a faster initial drug release for drugs with greater solubility in water.

The PLS results indicate that an increase in the number of fused aliphatic rings in the drug accelerates the burst. Such was not expected, as described above, there is an inverse correlation between the increase in the "amount" of aliphatic parts in a drug and its solubility in aqueous media. Hence, one would expect, an increase in aliphatic "parts" in a drug would decelerate the burst. An hypothesis could be that the fact that the thermodynamic balance is

reached sooner for aliphatic drugs, does not hinder the "speed" at which the transport occurs, mostly limiting the maximum amount of drug release during burst, $F_{B,in}$.

3.4. Exploiting the decision tree model for drug-carrier design

Given the good agreement between the identified impact factors and the literature, it seems possible to exploit the model for rational drug-carrier design. Taking as illustrative example the case of Risperdizone, a small drug molecule (410.5 Da) used in the test set, the decision trees shown in Figure 3-8 and Figure 3-9 can be used to predict the amount of drug released during burst ($F_{B,in}$) and the burst kinetics (k_b). The molecular descriptors and formulation characteristics are shown in Table 3-3. Risperdizone has an aliphatic atom count lower than 17, therefore starting from the "root" of the decision tree (on top of Figure 3-8), the left branch of the decision tree is followed. At the next node a design choice has to be taken, namely the molecular weight of PVA utilized as surfactant during particle manufacturing can be chosen. The PVA Mw used in the test experiment is lower than 22 kDa, therefore the left branch of the tree is followed. The next design choice concerns the encapsulation efficiency; once it is lower than 95% then the size of the particles will determine the amount of drug release during burst, i.e.,for particles smaller than 130 nm, the predicted amount of drug release during burst ($F_{B,in}$) is about 47%, whereas for particles greater than 130nm, the $F_{B,in}$ will be about 71%. The actual predicted value for $F_{B,in}$ utilizing the molecular descriptors of Risperdizone and the formulation characteristics of the test experiment (Figure 3-8) is 71% (see Figure 3-8), and the experimental $F_{B,in}$value is 63%. The same procedure is followed for the prediction of the k_b. Starting from the root of the decision tree (Figure 3-9): the encapsulation efficiency 89% is not lower than 14%, therefore the right branch is followed. The next decision refers to the number of oxygen atoms in the drug molecule: it is two, lower than three, therefore the left branch is followed. Finally the inverse of the size of the particle squared ($1/D^2$) is 2e4 μm^{-2}, lower than 6.5 μm^2, which determines the burst kinetics parameter, k_b, of 0.14 day^{-1}, and the experimental value is 0.15 day^{-1}. As this illustrative example shows, depending on the drug, different design choices can be made to control burst. For instance, for small drug molecules, the PVA Mw, the encapsulation efficiency and the particle size have an predominant impact on $F_{B,in}$ whereas for bigger molecules the PVA concentration (outer phase), the PLGA Mw and the particle size have the dominant effect on $F_{B,in}$. Thus the model enables a rational design focusing on the main factors rather than considering all possible impact factors. Note that changes in some variables for optimization of $F_{B,in}$, can also affect k_b. Therefore, the optimization of both properties should be carried out simultaneously.

Table 3-3. Molecular descriptors and formulation characteristics used in the decision trees for a test experiment with Risperdizone. Experimental (*exp*) and decision trees predicted (*pred*) values of $F_{B,in}$ and k_b.

O_{no}	Aliphatic atom count	PVA Mw, kDa	Encapsulation Efficiency, %	Particle size (D), µm	Particle size $(1/D^2)$, μm^{-2}	$F_{B,in}$ exp/pred, %	k_b exp/pred, day^{-1}
2	15	18	88.6	69	2e+4	63/71	0.15/0.11

3.5. Conclusions

In this work the impact of the formulation characteristics, the synthesis parameters and drug molecule descriptors on the amount and kinetics of burst drug release were analyzed by two distinct regression methods, namely PLS and decision trees. The predictive power of decision tree regression is shown to be significantly better than PLS. More importantly, the discrimination of input factors with a significant impact on the amount of burst release and respective kinetics by both methods is largely concordant. Both methods suggest that the amount of drug released during burst is mostly influenced by the formulation characteristics and the synthesis parameters, whereas the drug release kinetics is also influenced by the molecular properties of the drug. The variables that significantly influence the amount and kinetics of the release are discussed in detail and in general the results agree with those of other researchers. Additional impact factors might be found in the future when adding new experiments to the data set. Results that could not be corroborated were associated with the molecular descriptors of the drug, which cannot be studied experimentally. Also PVA molecular weight was found to have a significant influence on the amount of release yet its impact has not been studied experimentally. The molecular weight of polymers (PLGA and surfactants) was found to significantly influence both the amount and kinetics of burst release. While the mean molecular weights were used in the presented analysis, it was noticed that the range of molecular weights is relatively wide. In future it would be useful to either better characterize the polymer weight distribution (which could then be used in the analysis) or to reduce the range in molecular weights.

Due to the good agreement of the modeling results with the findings of other researchers as well as the validation and testing of the model structures, the models developed here and particularly the decision tree model can be used to design the carrier for a new drug in a way to minimize the burst release.

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3.6. Supplementary Material

Table 3-4. Description of variables of input for regression models.

Molecular descriptors of the drug Calculated using MarvinSketch [30]				**Formulation characteristics**	
1	C no	26	Hetero ring count	51	PLGA molecular weight
2	H no	27	Hyper wiener index	52	Lactide to glycolide ratio in PLGA
3	N no	28	Largest ring size	53	Use of PVA, yes/no (y/n)
4	O no	29	Largest ring system size	54	Use of TPGS (vitamin E), y/n
5	S no	30	Mass (molecular weight)	55	Use of poloxamer 188, y/n
6	Aliphatic atom count	31	Maximal projection area	56	Use of SDS (sodium dodecyl sulfate), y/n
7	Aliphatic bond count	32	Maximal projection radius	57	Use of DCM (Dichloromethane), y/n
8	Aliphatic ring count	33	Length perpendicular to the max area	58	Use of EtAc (ethyl acetate), y/n
9	Aromatic atom count	34	Minimal projection area	59	PVA conc inner phase
10	Aromatic bond count	35	Minimal projection radius	60	PVA conc external phase
11	Aromatic ring count	36	Length perpendicular to the min area	61	PVA molecular weight (Mw)
12	Asymmetric atom count	37	Platt index	62	Other surfactant Mw
13	Bond count	38	Randic index	63	Encapsulation efficiency, %
14	Carboaromatic ring count	39	Szeged index	64	Initial drug loading, %
15	Carbo ring count	40	Refractivity	65	Mean particle diameter, D
16	Chain atom count	41	Ring atom count	66	D^2
17	Chiral center count	42	Ring bond count	67	$1/D^2$
18	Cyclomatic number	43	Ring count	68	Use of PEG, y/n
19	Balaban index	44	Ring system count	69	PLGA concentration
20	Dreiding energy	45	Rotatable bond count	70	PLGA to PEG ratio
21	Fused aliphatic ring count	46	Atom count	71	w/o/w, water-in-oil-in-oil method, y/n
22	Fused aromatic ring count	47	Chain atom count	72	s/o/w, solid-in-water-in-water method, y/n

23	Fused ring count	48	Chain bond count	73	s/o/o, solid-in-oil-in-oil method, y/n
24	Heteroaliphatic ring count	49	van der Waals volume	74	o/w, oil-in-water method, y/n
25	Heteroaromatic ring count	50	pI, isoelectric point		

Legend: no=number of atoms in the drug molecule

3.7. References

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Chapter 4 - Hybrid model-based prediction of burst release as function of encapsulation method, carrier properties and drug properties: the case of PLGA micro- and nanoparticles

ABSTRACT

One of the major challenges for designing an efficient controlled drug release system is overcoming the initial burst release. The main objective in this study was the development of a model-based control method of the amount of drug released during burst. The method was applied to poly(lactic-co-glycolic) acid (PLGA) micro- and nanoparticles but can be easily extended to other delivery carriers. A hybrid model was developed describing the amount of drug released as function of the formulation characteristics, the synthesis parameters and drug molecule descriptors. The hybrid model combines the Corrigan model with artificial neural networks, the latter describing the dependency of Corrigan model parameters as functions of the designs parameters and drug parameters. The model was developed on 132 release profiles and its predictive capability was tested on a data set not used for model development. The hybrid model is shown to predict the test profiles with acceptable accuracy. It is further shown that the model describes the impact of design parameters on the drug release profile coherent with experimental observations. All in all this study shows that hybrid modeling is a powerful methodology that can aid the design of the carrier of a new drug to achieve desired drug release characteristics.

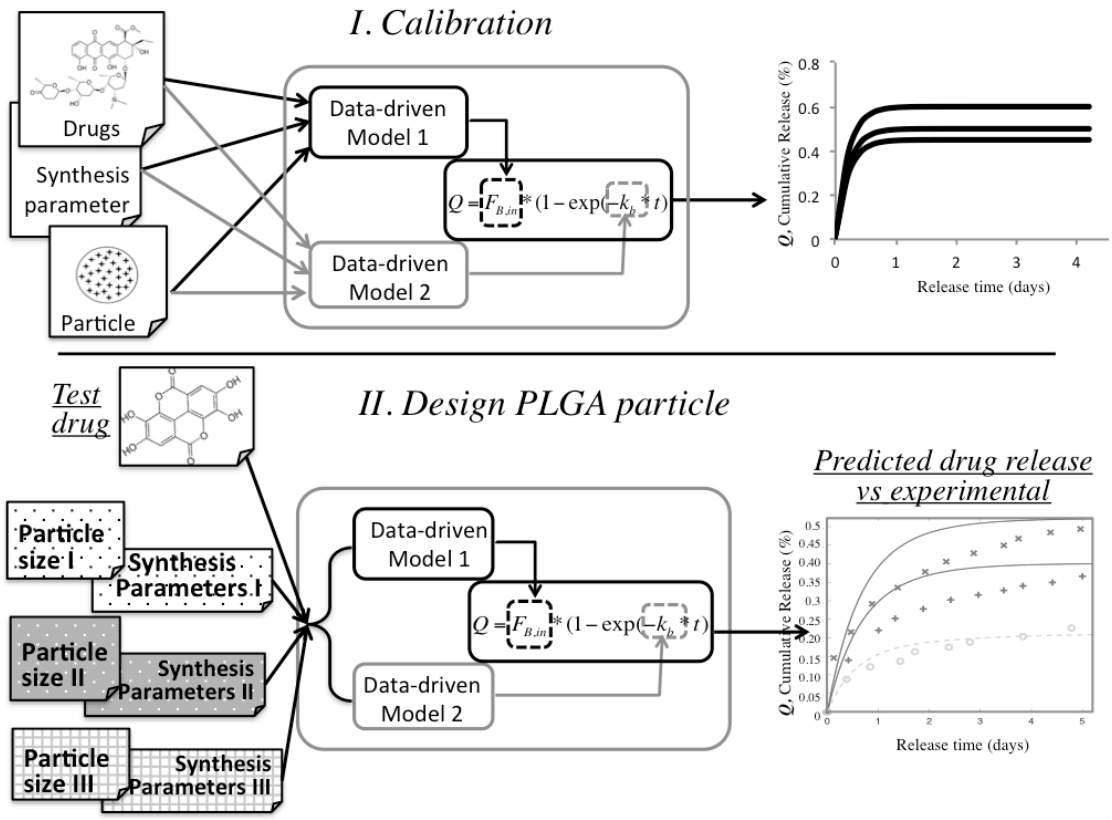


Figure 4-1. Graphical Abstract

Keywords

Controlled Drug Release, Burst release, Artificial neural networks, Hybrid model, PLGA carriers

4.1. Introduction

Substantial drug release from PLGA micro- and nanoparticles can occur during the first days of immersion, which is referred to as burst release [1-7]. Burst release has been attributed to a rapid desorption of the drug located on or near the surface of particles, and to poorly encapsulated drug, which rapidly diffuses immediately upon immersion into a biological fluid. Typically the burst release is an unwanted event, since the high initial drug concentrations can have negative effects on the host (see e.g., review of Huang et al. [8]). An unwanted burst release, usually leads to a shorter drug release time and obstructs cornerstones of controlled drug release, predictability and reproducibility. Although being a widely documented phenomenon [8-17], novel approaches are needed to prevent and/or control burst release.

Mathematical modeling has been used to describe and predict the controlled release profile of specific drugs. Several researchers have derived modeling approaches based on a mechanistic or phenomenological understanding of drug release from micro- and nanoparticles [18-27]. However, the experimental conditions and drug properties, which have a significant impact on the drug release, are rarely taken into account by these models. In some cases, the mean particle size is included, but the carrier formulation (e.g., monomers ratio, surfactants used during particle synthesis), which has a significant impact on the drug release, is not considered. Thus, the analysis of the impact of experimental conditions or drug properties on the drug release is not possible by these models.

More recently, data-driven methodologies have been applied to model controlled drug release from tablets [28] and from PLGA microparticles [29, 30] aiming at the correlation of the release profile with physicochemical properties of the carrier and/or drug. The utilized data-driven methods lack mechanistic interpretability and hence are many times regarded as black-box methods. Due to the black-box nature of such models, limited understanding is provided in relation to how the formulation characteristics or drug properties impact on the drug release. Also these models completely disregard valuable *a priori* knowledge of well-established drug release models available in the literature.

In a previous study (see Chapter 3) the impact of the formulation characteristics, the synthesis parameters and drug molecule descriptors on the amount and kinetics of burst drug release were analyzed using PLS and decision trees. However, the simulation of the cumulated drug release profile using the predicted amount and kinetics of burst drug release did not result into a good description, because the re-normalization (the normalization had been necessary to obtain normally distributed data for training of the models) of the prediction amplified prediction errors.

In this work, the burst release from PLGA particles is modeled utilizing a hybrid modeling (HM) approach which combines the burst release model proposed by Corrigan et al. [31] with Artificial Neural Networks (ANN). The role of the ANNs is nonlinear regression of Corrigan model empirical parameters as function of synthesis, particle design and the drug properties. The sensitivities approach [32] is applied to identify the neural network weights from the cumulated drug release profile, such reducing the impact of error amplification. PLGA micro- and nanoparticles were chosen as controlled delivery carrier in this work due to their widespread use [33]. Particular features are that: 1) PLGA is biodegradable and biocompatible; 2) it allows for a fine-tuning of its mechanical properties and consequent degradation rate; and 3) it is FDA approved in a range of several PLGA-based drug products [34]. Also a thorough review on modeling of CDR from PLGA microparticles can be found in Versypt et al. [35].

4.2. Materials and methods

4.2.1. A hybrid semi-parametric drug release model

4.2.1.1. The parametric backbone of the drug release model

The model proposed by Corrigan et al. [31,36,37] contains a term that explicitly describes the drug release during the burst phase. Due to its ability to describe a wide range of drug release profiles as well as its similarity to other models [6,37,38], this model and in particular the term describing the drug release during the burst phase, was chosen as the parametric backbone of the hybrid model:

$$\boldsymbol{Q = F_{B,in} \cdot (1 - \exp(-k_b \cdot t))} \quad \textbf{(1)}$$

where Q is the total fraction of drug released at a given time t, (a value between 0 and 1), $F_{B,in}$ is the fractional amount of the drug released during the burst and k_b a first order rate constant associated with the kinetics of the burst release. The values of $F_{B,in}$ and k_b will change depending on the drug, the formulation characteristics or the experimental conditions, wherefore the idea is to use data-driven models, namely ANNs, to describe these dependencies.

4.2.1.2. Artificial Neural Networks

Two Artificial Neural Networks (ANNs) are used to model $F_{B,in}$ and k_b as functions of the drug (represented by molecular descriptors), the formulation characteristics and the experimental conditions, because ANNs can approximate arbitrarily complex nonlinear functions [39]. The structure of the ANNs has to be determined from data, wherefore they belong to the class of nonparametric models. Here, ANNs with three layers, an input, output and hidden layer, were used, as three layers typically suffice to model complex nonlinear functions. Linear transfer functions were chosen for the nodes in the input and output layers, whereas hyperbolic tangential transfer functions were chosen for the hidden layers. The procedures to select the number of inputs and number of nodes in the hidden layers from the data as well as the data are described next.

4.2.2. Data

4.2.2.1. Database

The database from Szlęk et al. [29] was curated and extended with data from literature in total comprising data of 152 in vitro CDR experiments. The 152 experiments comprise 41 different active pharmaceutical ingredients (in this study for simplicity referred to as “drug”) that have wide therapeutic applications, as described in more details in in [40]. The database encompasses i) the cumulative drug release profiles over time (extracted from articles where

possible directly and else using the image recognition software Plotdigitizer (version 2.6.8)); ii) 50 molecular descriptors for each drug (calculated with the ChemAxon plugin from Marvin (v5.2.1 [41])); and iii) formulation characteristics of the drug-carrier synthesis, which were extracted from the protocols of the drug-loaded PLGA particles preparation methods, see [40] and Table 4-2 for more details.

4.2.2.2. Inputs selection

The input variables for the $F_{B,in}$ and k_b neural networks were selected from the 74 available variables choosing the eight most significant variables by the feature selection described in chapter 3. In case of $F_{B,in}$ the inputs are: (1) aliphatic atom count of the drug molecule, (2) PVA Mw, (3) PVA concentration on the outer phase, (4) encapsulation efficiency, (5) particle size D, (6) Balaban index and (7) PLGA and (8) initial drug loading.

In case of k_b the inputs comprise, (1) the encapsulation efficiency, (2) PLGA Mw, (3) number of oxygen atoms on drug molecule, (4) $1/D^2$, (5) PVA concentration on the inner phase, (6) isoelectric point, (7) PLGA to PEG ratio and (8) PVA Mw.

In theory, more input variables could be used if more data of drug profiles were available. The eight selected inputs (in each case) yield a good performing model while not over-parameterizing the system, i.e.,with the maximum tested number of six nodes in the hidden layer, the parameter/data points ratio was greater than 0.5.

4.2.2.3. Data Partitions

One part of the data, 20 profiles, was allocated as test partition. Since the number of the remaining drug profiles was relatively low a bootstrap aggregated model strategy was adopted [42]. Thus, the remaining data was randomly partitioned ten times into a training (80% of the points) and a validation (20% of the points) partition, i.e.,ten training-validation data sets were prepared. For each set the neural networks were trained on the training partition, and the validation partition was used to stop the training, i.e.,cross-validation. The test partition was then used to evaluate the performance of the final aggregated model as described below.

4.2.3. Parameter Identification and Structure Discrimination

4.2.3.1. Parameter Identification

A two level identification procedure was used for determining the weight values of the neural networks.

Level 1: At first, the parameter values of $F_{B,in}$ and k_b were estimated for the drug profile contained in the training-validation set using the Matlab function “lsqnonlin”, which uses the Marquardt-Levenberg method for minimization of a nonlinear least squares objective function. In order to estimate the confidence intervals of the parameters, Monte Carlo

sampling (100 repetitions) was used on the experimental data assuming standard error of 2.5% of the experimental value. It is considered that no drug was released at the beginning of the experiment $Q(t = 0) = 0$).

Level 2: Once the $F_{B,in}$ and k_b values had been estimated they were used to determine the weight values of the neural networks. In case of $F_{B,in}$ the values were auto-scaled, using the mean and standard deviation values of the training-validation partition (which are identical for all data sets). The auto-scaled values of the training partitions were then used to minimize a least square function employing a Marquardt-Levenberg method, i.e., the Matlab function "lsqnonlin". For each training partition the weight values of one neural network with varying number of hidden layers were identified. In case of k_b the natural logarithm of the values was calculated first to account for the significant differences in magnitude. The log-treated values were then auto-scaled, again using the mean and standard deviation values of the training-validation partitions. Then the same procedure as for $F_{B,in}$ was followed. The training was stopped in both cases when the mean-squared error in the validation partitions did not decrease further. The identification for both $F_{B,in}$ and k_b was started 400 times from random weight values to avoid getting stuck in local minima, and the best performing iteration (in terms of MSE calculated for the validation set) was chosen.

4.2.4. Model Structure Discrimination

For each of the ten sets and for both neural networks the number of nodes in the hidden layer was varied systematically between one and six, and for each structure the best performing parameter set was chosen. Thus, six networks with varying number of hidden nodes were obtained for each parameter $F_{B,in}$ and k_b and for each of the ten sets. The best performing structure for each parameter and each set was chosen according to the Akaike Information Criterium (described below). For each set, the best performing neural networks for $F_{B,in}$ and k_b were then used together with the parametric model to calculate the drug profile Q and compare its fit with the experimental drug profiles. The HM calculated drug profiles Q for all ten sets were then averaged to establish the final aggregated model.

4.2.5. Criterion for Model Performance

Model performance criteria are used to assess the performance of the Neural Networks and the aggregated hybrid model. In terms of cost function minimization, several authors have adopted the Akaike Information Criterion (AIC) for the selection of the hybrid model structure. The AIC is based on the Kullback-Leibler (K-L) information loss, which may be conceptualized as a "distance" between the true model and the approximating candidate model [43]. In practice, the AIC with second order bias correction ($AICc$) is preferred over

AIC since AIC tends to select models that overfit for small samples while $AICc$ is valid for both small and high number of samples (n). The $AICc$ takes the following simplified form:

$$AIC_c = n\ \ln\left(MSE\right) + 2k + \frac{2k(k+1)}{n-k-1} \quad \textbf{(2)}$$

where k is the number of estimated parameters in the model [44]. The lower the value of $AICc$, the better is the adjustment of the model to the reality.

The mean squared error (MSE) is a widely used qualitative measure to evaluate the fit of the model predictions with the experimental data. Its calculation is based on the average squared distance between the modeled and the measured values, i.e.:

$$\boldsymbol{MSE = \frac{1}{n}\sum_{i=1}^{n}(y - y_{mes,i})^2} \quad \textbf{(3)}$$

where y represents either the values of $F_{B,in}$ or k_b in case of the individual ANNs, or the cumulative drug release Q when analysing the regression quality of the HM.

4.3. Results and Discussion

4.3.1. Model Structure Discrimination

The neural networks for each ten data sets were individually trained for $F_{B,in}$ and k_b. The number of nodes in the hidden layer of the neural networks, which minimize the $AICc$ for each set are shown in.Table 4-1 Across the ten sets the performance of the HMs were similar, i.e.,the number of hidden nodes that minimize the $AICc$ vary between 3 and 6, 4 being the most frequent. For both $F_{B,in}$ and k_b the performance of the networks in terms of MSE and $AICc$ are comparable.

Table 4-1: Selection of the best performing network structures for $F_{B,in}$ and k_b in terms of AICc calculated for the training-validation data for each of the ten partitions. The MSE performance is also shown.

No. of Set	Number of Hidden nodes	MSE, mean	AICc
1 $F_{B,in}$	5	0.0101	-2791
1 k_b	4	0.0045	-3320
2 $F_{B,in}$	5	0.0089	-2871
2 k_b	6	0.0022	-3821
3 $F_{B,in}$	6	0.0081	-2908

3 k_b	4	0.0027	-3623
4 $F_{B,in}$	3	0.0134	-2767
4 k_b	4	0.0038	-3447
5 $F_{B,in}$	4	0.0122	-2683
5 k_b	4	0.0028	-3619
6 $F_{B,in}$	4	0.0116	-2704
6 k_b	4	0.0036	-3461
7 $F_{B,in}$	4	0.0118	-2709
7 k_b	4	0.0029	-3623
8 $F_{B,in}$	4	0.0100	-2818
8 k_b	5	0.0033	-3576
9 $F_{B,in}$	5	0.0076	-2670
9 k_b	6	0.0027	-3766
10 $F_{B,in}$	4	0.0093	-2805
10 k_b	4	0.0034	-3487

4.3.2. Bootstrap Aggregated Hybrid Model Performance

Using the identified ANNs for $F_{B,in}$ and k_b in conjunction with the Corrigan equation, a hybrid model is obtained. The values of Q obtained by each of the ten HMs (with the ANNs structure specified in Table 4-1) were aggregated. The modeled cumulative release values against the experimental are shown in

Figure 4-2. It can be seen that the model generally fits the measured values well for both the training-validation and test set. The obtained MSE values for the training-validation and test sets are 0.005 and 0.037, respectively. The slightly greater MSE value of the test set is mostly due to some outlier points that can be seen at the lower right side of Figure 4-2. These points describe bovine serum albumin (BSA) release experiments. The reason for the inferior model performance might be that BSA is a relatively large molecule (66.5 kDa), wherefore different mechanisms might occur in the experiment than in the ones used for creating the models. In future, it would be useful to not only report findings in form of articles, but also share the data and adopted experimental methods in a public database. This could significantly improve the

quality and availability of data for the creation of predictive controlled drug release hybrid models, as it would become e.g., possible to include more characteristics and descriptors in the models.

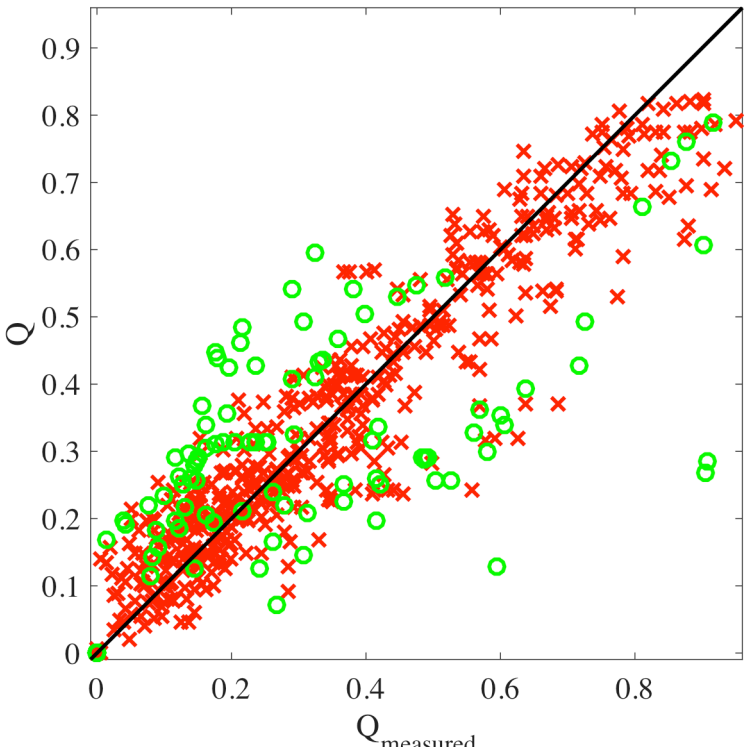
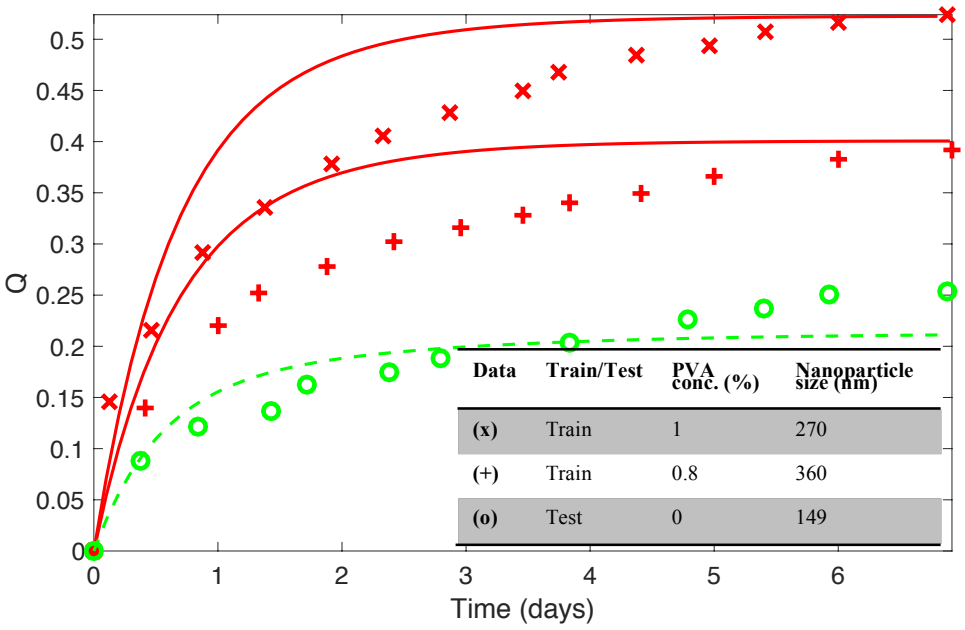


Figure 4-2: Bootstrap aggregated hybrid model prediction of drug release values (*Q*) versus measured values of cumulative drug release ($Q_{measured}$). Red crosses: training set, green circles: test set.

4.3.3. Analysis example of the impact of particle synthesis parameters on drug release profiles

A representative example of simulated and measured cumulative drug release over time is shown in Figure 3-5 for the case of ellagic acid [45]. The red profiles are from the training-validation set, whereas the green are from the test set. Different concentrations of PVA for particle synthesis, as well as nanoparticles of different sizes were used in the three experiments, see insert of Figure 3-5 for details. It can be seen in Figure 3-5 that the agreement between simulated and experimental values is good. In particular, the variations in the release profiles which are due to variations in nanoparticle size and PVA concentration are well described by the model and consistent with the observations from the experiments. This is a good indicator that the application of the model for particle design and formulation is possible.



Data	Train/Test	PVA conc. (%)	Nanoparticle size (nm)
(x)	Train	1	270
(+)	Train	0.8	360
(o)	Test	0	149

Figure 4-3: Comparison of predicted (continuous lines) and experimental values (markers) of cumulative burst release of ellagic acid along time [45]. Data shown in red are part of the training set, and data shown in green are part of the test set.

4.4. Conclusions

In this work drug release profiles are modeled via the Corrigan equation in which changes in its parameters associated with the total amount of drug released during burst and the burst kinetics are described by neural networks. The ANNs were derived as functions of the synthesis parameters and molecular descriptors of the drug. A bootstrap aggregating identification strategy was used for the development of the hybrid model. After development, the model was used to predict the cumulative drug release of an independent set of CDR experiments. Good agreement between the predicted and the experimentally measured cumulative drug release profiles was observed. On an example it was shown that the HM accurately describes the impact of changes in synthesis parameters on the release profile of ellagic acid. The use of more and also more qualitative data could increase the performance of the model in the future. Sharing of the experimental data and accurate reporting of the associated methods in a database could therefore greatly help to exploit the full capabilities of the hybrid modeling methodology. All in all, the performance of the developed hybrid model is acceptable and it can be used for optimal drug-carrier design by manipulation of synthesis parameters and formulation characteristics.

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4.5. Supplementary Material

Table 4-2. Drug names and source of data utilized in this study

Drug designation	Abbreviation	Source
(1-Naphthalenyl[4-(pentyloxy)-1-naphthalenyl]methanone)	CB13	[46,47]
Aclacinomycin	ACM	[47]
Alpha-1 Antitrypsin	α1AT	[48]
Alpha-Chymotrypsin	AChT	[49]
Amoxicilin	AMX	[50]
Amphotericin B	AmB	[51]

Amyloid beta	$A\beta_{1-15}$	[52]
Bovine insulin	B-INS	[53]
Bovine serum albumin	BSA	[54-57]
Camptothecin	CPT	[58]
Cisplatin	CIS	[59]
Clonazepam	CLZ	[60,61]
Curcumin	CUR	[62]
Daunorubicin	DAU	[47]
Dexamethasone	DXM	[11]
Doxorubicin	DOX	[47]
ellagic acid	EA	[45]
Epirubicin	EPI	[47]
Estradiol	EST	[13]
Etoposide	ETO	[64]
Exenatide (synthetic exendin-4)	EXE	[65]
(5-)Fluorouracil	5-FU	[66]
Gamma-chymotrypsin	GChT	[67]
Human serum albumin	HSA	[31]
Idarubicin	IDA	[47]
Indomethacin	IND	[31]
Insulin	INS	[68]
Ketoprofen	KET	[31]
L-asparaginase	L-ASP	[69]
Lysozyme Recombinant Protein	LZM	[54]
Minocycline	MIC	[70]
Nalmefene	NAL	[71]
Ovalbumin	OVA	[31]
Paclitaxel	PTX	[72,73]
Quercetin	QCT	[74]

Recombinant Human Epidermal Growth Factor	rhEGF	[5]
Recombinant human erythropoietin	rhEPO	[75]
Risperidone	RIS	[76]
Ropivacaine	ROP	[77]
Tumor necrosis factor receptor	OX40	[78]
Vincristine	VCR	[58]

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Chapter 5 - A methodology for rational PLGA carrier design minimizing burst release with application to Activin A and α-chymotrypsin

Abstract

A burst of drug release is known to occur in the initial hours of nanoparticle immersion in a medium. This initial release presents a challenge for a sustained and predictable controlled drug release system. To date the design of nanoparticles consider the burst release at a limited degree, being governed by heuristics and trial and error methods.

In this paper, a hybrid model-based optimization platform is developed to aid the design of drug-loaded Poly-lactic-co-glycolic-acid (PLGA) nanoparticles for a controlled initial burst. The optimal formulation characteristics are determined by minimizing the difference between bootstrap aggregated hybrid model predictions and a desired burst release profile while also considering the uncertainty of the model predictions. The methodology is applied for the carrier design of α-chymotrypsin. A nanoparticle design was obtained that yields a close to desired release profile and at the same time low prediction uncertainty. The impact of changes in the formulation characteristics on the release profile was further investigated by a sensitivity analysis. Subsequently, the burst release predictions of the α-chymotrypsin were then compared to those of Activin A using the particle design of α-chymotrypsin. The proteins have similar characteristics, in terms of the key molecular descriptors, and show similar predicted burst release profiles. All in all these results are encouraging to experimentally explore the working space of acceptable formulation characteristics with drug replacements, which are typically more economical, providing additional data to further improve the model.

Keywords

Controlled Drug Release, Burst release, aggregated Hybrid model, PLGA nanoparticle, mock-protein

5.1. Introduction

The design of the optimal biodegradable carrier for a drug is still dominated by the use of rules of thumb and a great deal of trial and error experimentation [1,2]. Design choices have to be made for a number of variables that are known to impact the amount of drug release and its kinetics during the different stages of a controlled release experiment. The impact of physicochemical parameters of the carrier and molecular descriptors of the drug on the different stages of drug release from PLGA microparticles was reviewed in [2,3] However, the impact of the variables is not quantified, as it might be case dependent. Also only the impact of one variable at a time is described in these reviews and the interdependencies between variables were neglected [4], which implies that it is not clear what will happen to the drug release when changing e.g., PLGA molecular weight and surfactant concentration at the same time. As shown by de Azevedo et al. [5] (chapter 3), interdependencies are common for many design parameters and they proposed a model that can predict the drug burst release when manipulating several variables at a time.

Burst release has been identified as the major challenge for a sustained and controlled drug release from micro and nanoparticles [3], wherefore this work is focused on the design of nanoparticles for control of the burst release. Burst release can occur in the initial hours of immersion of drug-loaded nanoparticles, releasing a significant amount of drug during a short period. The magnitude and kinetics of the burst release can be tuned by manipulating key variables such as the initial drug loading, the encapsulation efficiency and the particle size [2–4,6–11].

Recently de Azevedo and co-workers developed a (bootstrap aggregated hybrid) model that links the drug molecular descriptors and formulation characteristics to the burst drug release from PLGA particles. The hybrid model comprises a mechanistic drug release model [12] (equation 1) where the changes in the amount of drug release and the burst kinetic parameters with changes in the formulation characteristics and molecular descriptors are described via two Artificial Neural Networks (ANN) [[5], chapter 3]. It was shown that the model could, in principle, be used for rational drug-loaded carrier design in chapter 4. Here, this hybrid modeling approach is applied for the optimization of the PLGA-carrier design to reduce/control the initial burst release.

In this paper an optimization approach is proposed that designs the nanoparticle for a given drug and desired drug release profile, while also considering the uncertainty of the model predictions. The approach is applied to two case studies. In the first case, the design choices and the sensitivities are analyzed for nanoparticles encapsulating a mock protein (α-chymotrypsin) that mimics a target drug, Activin A (which is relatively expensive). In the

second case, the predicted profile of the mock protein is compared to that predicted for Activin A using the formulation characteristics found to be optimal for the mock-protein.

5.2. Material and Methods

5.2.1. A rational particle design approach

A framework for the rational design of a PLGA nanoparticle given a desired burst release profile is proposed here. A schematic representation of the proposed design approach is shown in Figure 5-1 The workflow is as follows: i) the molecular descriptors are calculated for the given drug and fixed, whereas limits (lower and upper bounds, based on the range seen in the database used to train the aggregated hybrid model) are provided for the formulation characteristics, as they are manipulated during the optimization; ii) the molecular descriptors of the drug and the formulation characteristics are inputs to the bootstrap aggregated hybrid model that predicts the average cumulative drug release ($\overline{Q_{pred}}$); iii) a numerical optimization algorithm is used to minimize the difference between the $\overline{Q_{pred}}$ and Q_{des} by manipulation of the formulation characteristics. The optimization continues until the improvement is below a certain threshold, ε.

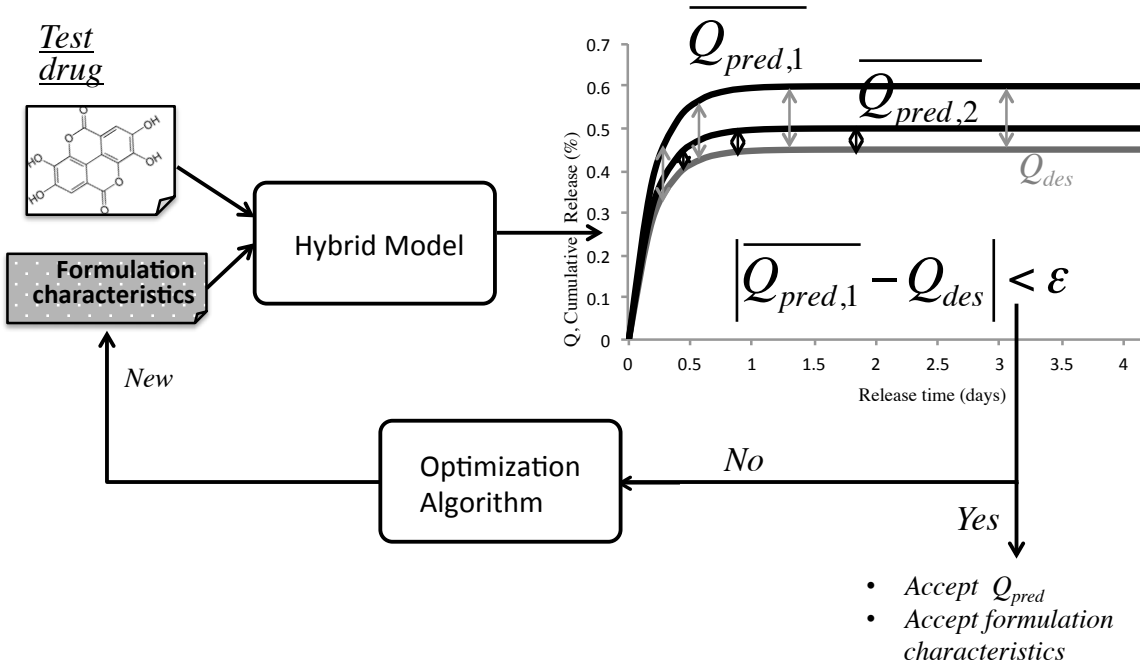


Figure 5-1.Schematic representation of the PLGA nanoparticle design approach, given a desired initial burst release profile and drug.

5.2.2. Hybrid Model

The previously developed bootstrap aggregated hybrid model (chapter 4) was utilized for model-based design. The model combines several hybrid models (by averaging their predictions, $\overline{Q_{pred}} = \sum_{n=1}^{N} Q_{pred,n} / N$, with N equal to the number of hybrid models, $Q_{pred,n}$ is the cumulative fraction of drug released at a given time t predicted by each individual hybrid model), where each hybrid model was developed and validated on a different subset of the data. Each of the hybrid model combines the burst release model proposed by Corrigan et al. [12]:

$$Q_{pred,n} = F_{B,in} \cdot (1 - \exp(-k_b \cdot t)) \quad (1)$$

with two ANNs describing the changes of fractional amount of the drug released during the burst and the kinetics of the burst release ($F_{B,in}$ and k_b, respectively) as function of synthesis, particle design and drug descriptors. In particular, the inputs of the ANNs comprise the formulation characteristics as well as drug molecular descriptors found to be of impact on burst release from PLGA particles in previous work [2]. In case of $F_{B,in}$, these are the aliphatic atom count of the drug molecule, PVA Mw, PVA concentration on the outer phase of particle synthesis, encapsulation efficiency, particle size, Platt index and PLGA Mw and initial drug loading. In the case of k_b the inputs comprise, the encapsulation efficiency, PLGA Mw, number of oxygen atoms on drug molecule, PVA concentration on the inner phase of particle synthesis, protein isoelectric point and PVA Mw. The procedure for development and testing of the hybrid model was described before (chapter 4).

5.2.3. Optimization problem

The optimization aims at minimizing the difference between Q_{des} and $\overline{Q_{pred}}$ by manipulating the degrees of freedom, i.e., the seven formulation characteristics, u_{fc} (the encapsulation efficiency, initial drug loading, PVA concentration during PLGA particle synthesis (inner and external phase) particle size, PVA and PLGA Mw). Though upper and lower bounds exist and are defined for the formulation characteristics, similar differences might be obtained for very different values of formulation characteristics. Since the adopted modeling approach is partially data-driven, the prediction of the models can be expected to be the more accurate the closer the optimized formulation characteristics are to those characteristics under which the models were developed on. In order to account for this, those formulation characteristics for which all of the aggregated models predict similar release profiles are promoted, i.e., the standard deviation between the predicted release profiles should be low. Minimizing both properties at the same time the objective function is as follows:

$$\min_{u_{fc}} \left\{ \sum_{t_{burst}} \left[\boldsymbol{w} \cdot \left(Q_{des} - \overline{Q_{pred}} \right) \right]^2 + \lambda \cdot \sum_{t_{burst}} \sqrt{\frac{\sum_{n=1}^{N} \left(Q_{pred,n} - \overline{Q_{pred}} \right)^2}{N-1}} \right\} \quad (2)$$

A weight profile, $\boldsymbol{w}$, of dimensions equal to the number of time points was included in order to assign different weights to different stages of the burst release. This allows to enforce the minimization of the difference between $\overline{Q_{pred}}$ and Q_{des}, at particular time points, e.g., in order to assure that the total amount of drug release ($F_{B,in}$) is achieved towards the end. Hence, the weight profile $\boldsymbol{w}$ was composed in such a way to penalize more heavily differences between the $\overline{Q_{pred}}$ and Q_{des} in the final time points. The second term considers the variation in Q_{pred} and the parameter λ is used to balance the trade-off between the standard deviations of the predictions and the difference between $\overline{Q_{pred}}$ and Q_{des}. The optimization was solved using at first a global optimizer, namely the Matlab *ga* function with default conditions, and second a local optimizer to refine the solution, namely Matlab *fmincon* function with default conditions.

5.3. Results and Discussion

5.3.1. Case Study 1: Mock Protein

The pharmaceutical entity of interest in this work is Activin A. Activin A is a protein involved in the regulation of multiple biological processes, including cell differentiation and proliferation inflammation, neural development, and haematopoiesis [13–15]. Due to the high commercial value, a de-risking strategy for drug-carrier design was sought, where the initial release experiments would be performed with a "similar" protein assuming that the Activin-loaded nanoparticles will have a similar behaviour in terms of burst release, which will be studied in the second case study.

Based on the analysis which molecular descriptors of the drug are key to describe the burst release (chapter 3), the aliphatic atom count, number of carbon atoms, Platt index and the isoelectric point were chosen for comparing Activin A with the mock protein, α-chymotrypsin. The α-chymotrypsin was found to exhibit close similarities with Activin A (Table 5-1) and to be less costly, wherefore it is used as an economic representative protein, i.e., the mock protein.

Table 5-1. Activin A and α-chymotrypsin molecular descriptors values.

Parameter/ Variation	Number of carbons	Aliphatic atom count	Platt index	Isoelectric point	Molecular weigh, kDa
Activin A	1134	10	5004	9.38	26
α-chymotrypsin	1127	9	5010	10.12	25.7
Studied Range*	*4-6807*	*0-28*	*24-12820*	*0-12*	*0.13-81*

* Minimum and maximum values for the parameters in the database used to train the aggregated hybrid models

5.3.2. Optimization of the carrier system design

A desired release profile along time was defined according to pharmaceutical specifications (

Figure 5-2, black circles). Bounds for the initial loading and size of the nanoparticles were set due to experimental restrictions. The expected initial loading achieved has an upper bound of 0.05 % and the nanoparticle size requested due to transport requirements was bounded between 350 to 450 nm.

5.3.3. Analysis of the variations in the trade-off parameter λ

The impact of the trade-off parameter λ on the $\overline{Q_{pred}}$ and its standard deviations as well as on the corresponding optimal formulation characteristics was analyzed. The λ values of 0, 0.1, 0.2 and 0.5 were studied. The corresponding $\overline{Q_{pred}}$ and standard deviations are shown in Figure 5-2 and the respective formulation characteristics are shown in Table 5-2.

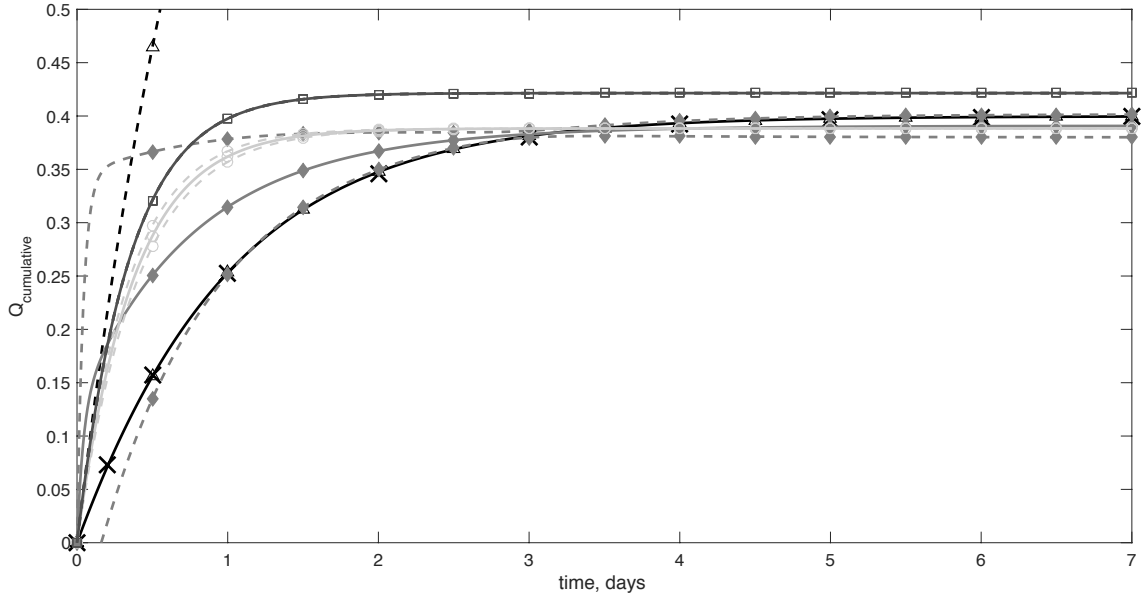


Figure 5-2. Cumulative desired release profile, Q_{des} (X), predicted release profile $\overline{Q_{pred}}$ (continuous line) and the respective standard deviations of the ten hybrid models (dashed lines) with: λ=0, triangles; λ=0.1,

diamonds; λ=0.2, circles; λ=0.5, squares. Standard deviations of the simulation with λ=0 are mostly outside of the plotted region (going towards -0.6 and 1.4) and are not shown to provide a higher resolution of the region of interest.

Table 5-2. Formulation characteristics corresponding to different λ values.

λ	Encapsulation Efficiency, %	Initial Loading, %	PVA conc, inner, %	PVA conc, extern, %	Size, µm	PVA Mw, Da	PLGA Mw, Da
0	89.35	0.03	0.51	0.07	0.12	172.64	63.91
0.1	11.42	0.03	2.14	2.12	0.38	65.47	199.83
0.2	**56.59**	**0.04**	**4.00**	**5.50**	**0.04**	**67.83**	**119.86**
0.5	7.63	0.03	1.65	4.96	0.08	36.19	140.90
Range*	*1-100*	*0.01-100*	*0-4*	*0.1-8*	*0.02-200*	*2-200*	*2-200*

* Minimum and maximum values for the parameters in the database used to train the bootstrap aggregated hybrid models

With increasing values of λ from 0 to 0.5, $\overline{Q_{pred}}$ moves away from Q_{des}, with λ =0, $\overline{Q_{pred}}$ matching the Q_{des} profile perfectly. Inversely, increasing λ values lead to a decrease in standard deviations, from a very wide standard deviation for λ =0, to a very narrow standard deviation for λ =0.5. The trade-off value of λ =0.2 was considered to provide a good trade-off between minimization of the prediction variation and match to the desired release profile. The corresponding formulation characteristics are referred to as baseline. These baseline formulation characteristics make sense from an experimental point of view; high PLGA Mw, with low degradation time is known to delay burst [4,5]. Encapsulation efficiency in the range of 60% is also quite good in terms of drug entrapment, leading to less drug being available for immediate release upon immersion [4]. The PVA concentration in the inner phase is different than zero, suggesting a double emulsion method for the preparation of the nanoparticles.

5.3.4. Sensitivity results

The sensitivity of the predicted release profile to changes in the formulation characteristics was investigated by varying the formulation characteristics. Exploratory experiments were designed around the baseline. In each analysis, the formulation characteristic in question is varied between the ranges of values shown in Table 5-3 in ten steps while the other six formulation characteristics are kept constant, with values equal to the baseline. The ranges of

PVA concentration (during PLGA synthesis, external phase) and initial drug loading investigated for sensitivity are narrower than the ones shown in Table 5-2 due to experimental constraints of the working space.

Table 5-3. Baseline parameter and formulation characteristics ranges.

Parameter/ Variation	Encapsulation Efficiency, %	Initial Loading, %	PVA conc, inner, %	PVA conc, extern, %	Size, µm	PVA Mw, kDa	PLGA Mw, kDa
Baseline	56.59	0.04	4.00	5.50	0.04	67.83	119.86
Range*	*1-100*	*0.01-40*	*0-4*	*4-8*	*0.02-200*	*2-200*	*2-200*

*Minimum and maximum values of the formulation characteristics used in the sensitivity analysis

The impact of changing each formulation characteristic on the predicted cumulative release is shown in Figure 5-3. Variations in the formulation characteristics can impact differently on the amount of drug released during burst and the burst kinetics, i.e., the "final" burst release is achieved at different times. This is of particular interest because it allows (at least to some degree) manipulating the kinetics independently of the amount of burst and vice versa.

It can be seen that most formulation characteristics are nonlinearly related to the cumulative release. The results presented in Figure 5-3 also make sense in light of the known rules of thumb of drug release. For instance, in the case of the encapsulation efficiency and PLGA Mw, an increase in their values, leads to less steep drug releases. High values of encapsulation efficiency are associated with a fast solidification of the polymer during particle formation, where the majority of the drug is entrapped in the interior of the matrix of the particle, unavailable for immediate diffusion upon hydration [3–5,16]. For PLGA, high molecular weight polymers are less soluble in the organic solvent used during particle synthesis, undergoing a fast solidification to produce particles with less porous, having therefore less channels available for drug release [6,17–21]. In terms of particle size, it can be observed that an increase of the particle size in the range of nanoparticles and small microparticles leads to a decrease of the burst release. This matches with the heuristic that the drug release is inversely proportional to the size of the nanoparticles, i.e., due to their larger surface to volume ratio the diffusion of drug released to the medium is increased [2,18,22]. On the other hand, in the realm of microparticles (>100 µm), the inverse was observed, where an increase of the particle size lead to an increase of burst. Such might be attributed to larger particles being more prone to erosion, and subsequent hydration, facilitating the drug diffusion from the polymer matrix interior of the microparticle [23]. For the impact of PVA concentrations (inner and external phase) during particle synthesis conflicting observations

have been reported [22,24], hence is difficult to infer general rules. Most authors assign an indirect impact to the PVA concentration to the drug release, since it directly affects the viscosity of the medium, the encapsulation efficiency and the particle size, depending heavily on the drug being encapsulated [4,22,24,25]. As can be seen in Figure 5-3, the burst release decreases with an increase of the PVA concentration in the inner phase until a threshold of 0.5%, followed by an increase, plateauing after 2%. Similar findings were observed by Yang et al. [24]. For PVA concentration in the external phase (Figure 5-3), an increase of PVA concentration until a threshold of 6.5% leads to an increase of the burst. The increase of PVA concentration in the external phase is associated with the formation of smaller particles [24]. In this case study with the baseline conditions, given the nanoparticle range, PVA concentration in the external phase leads to an increase in burst release. The impact of variations of the PVA Mw in the burst release has not been reported before, to the best of the authors' knowledge. It is predicted that increases of PVA Mw until a threshold of 20kDa slightly decreases the burst release, followed thereafter by an increase. Lyoo et al. [26] observed that an increase of the PVA Mw decreases the degree of its solubility in water, which might affect the concentration of drug in the aqueous phase during particle synthesis. For an increase of the initial drug loading until a threshold of 20%, an increase of the burst is predicted. Such is in agreement with several authors who associated increases of initial loading with more drug available (specially at the particle surface) to be diffused to the release medium [18,27–30]. A decrease of the burst release intensity was predicted for initial loadings greater than 25%. Such high initial loading (e.g., the present case study deals with very low initial loadings in the range of 0.04-0.05%) can be associated with very high encapsulation efficiencies, which are know to exhibit low burst releases [4]. A more detailed analysis of these formulation characteristics can be found in chapter 3.

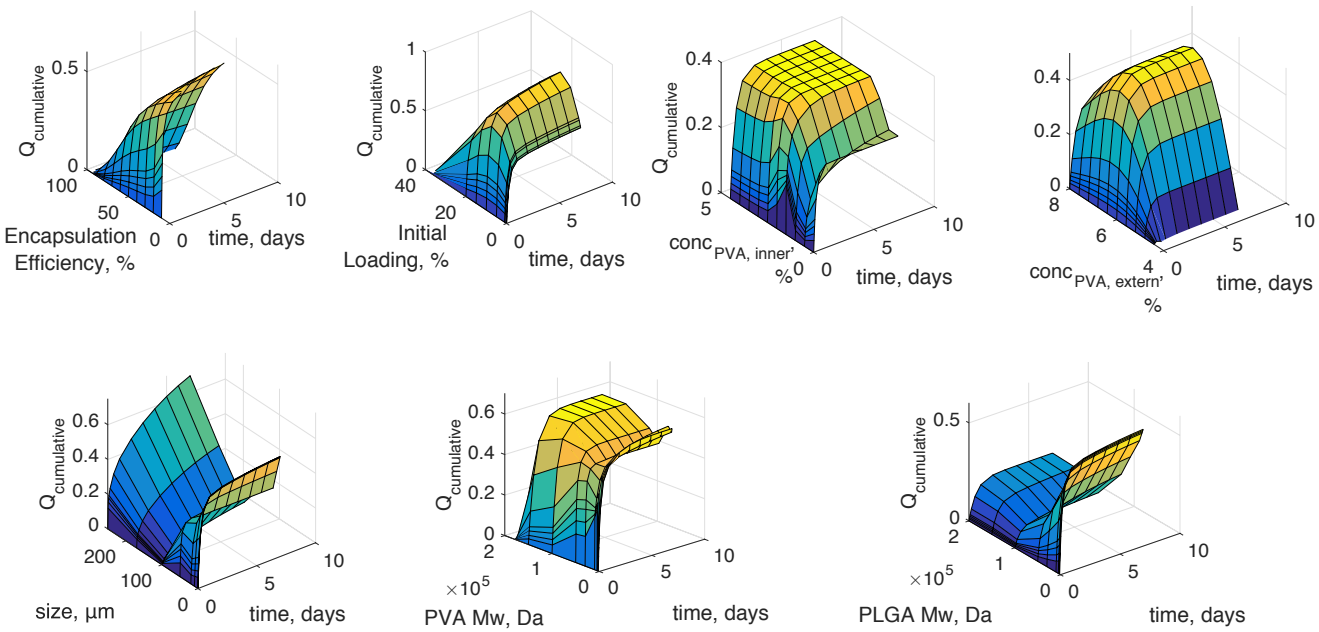


Figure 5-3: Surface plot of the bootstrap aggregated hybrid model predictions of the cumulative burst release versus input variable variations and time.

As stated before, the bounds of initial drug loading and size of the nanoparticles account for experimental restrictions. The expected initial loading has an upper bound of 0.05 % and the nanoparticle size was bounded between 350 and 450 nm. The impact of variations in both of these variables on the predicted cumulative drug release within the specified bounds is shown in Figure 5-4. Within the studied bounds of these variables, no significant impact of the variables on the prediction of the cumulative release can be observed. Therefore, certain experimental restrictions reduce the working space available to manipulate the response, i.e., the burst release.

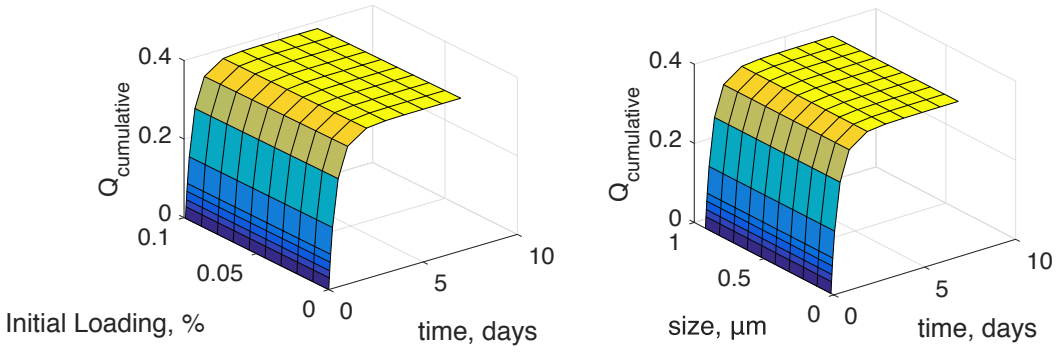


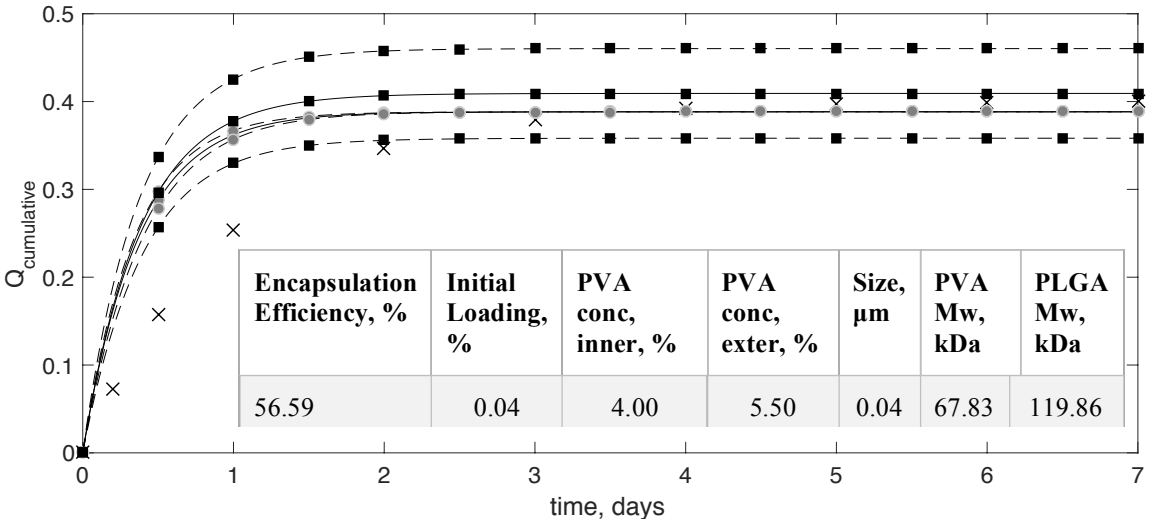
Figure 5-4. Predictive cumulative burst release simulated by aggregated hybrid model with restrictions: a) nanoparticle size and b) initial drug loading.

5.3.5. Case Study 2: Activin A

The above analysis conducted on the economic representative protein, α-chymotrypsin, was performed under the assumption that in fact the protein represents the behavior of Activin A.

Both proteins are similar in terms of those molecular descriptors, which are inputs for the aggregated hybrid model (Table 5-1) and thus important for describing the drug release. In this section, the baseline formulation characteristics determined for α-chymotrypsin (Table 5-3) were utilized to calculate the aggregated hybrid model predictions of the cumulative burst release ($\overline{\mathrm{Q_{pred}}}$) from nanoparticles loaded with Activin A.

The $\overline{\mathrm{Q_{pred}}}$ for both proteins match the above-specified $\mathrm{Q_{des}}$ closely (Figure 5-5). As was observed before, the standard deviations for the $\overline{\mathrm{Q_{pred}}}$ of α-chymotrypsin are very narrow (Figure 5-5, black lines), whereas wider standard deviations were obtained for Activin A. The width of the standard deviations could be reduced by optimizing the formulation characteristics specifically for Activin A, in particular by changing the nanoparticle size and the PLGA Mw. The good agreement between the $\overline{Q_{pred}}$ of both drugs with the same formulation characteristics is a good indicator that the mock drug-loaded nanoparticles can be applied at least as preliminary experiments to test the burst release profile of a more expensive pharmaceutical entity as function of the nanoparticle design. The data which would become available through such an experiment might help to re-train the model, potentially increasing the prediction reliability for the drug of interest, i.e., reducing the width of the standard deviations.



Encapsulation Efficiency, %	Initial Loading, %	PVA conc, inner, %	PVA conc, exter, %	Size, µm	PVA Mw, kDa	PLGA Mw, kDa
56.59	0.04	4.00	5.50	0.04	67.83	119.86

Figure 5-5. Averaged cumulative predicted burst release profile, $\overline{Q_{pred}}$ for α-chymotrypsin (grey continuous line and circles) and for Activin-A (black continuous line and squares) for nanoparticles with the same formulation characteristics. The lower and upper bounds of the standard deviation obtained from the predictions of the ten hybrid models are shown in dashed lines.

5.4. Conclusions

In this chapter the two PLGA nanoparticles each encapsulating a different protein were designed in such a way that a pre-defined burst release profile is achieved. The bootstrap aggregated hybrid model developed in chapter 4 was exploited for predicting the initial burst release when manipulating the formulation characteristics of the particle design to achieve the desired profile.

The proposed rational design method was applied to a mock protein (α-chymotrypsin), which exhibits similarities with the expensive drug, Activin A, in terms of important molecular descriptors with the target drug.

The design approach allowed choosing the formulation characteristics that matched the desired profile while also showing low standard deviations between the predictions of the aggregated models.

The sensitivity analysis was performed for the optimal formulation characteristics and the results, when compared to literature findings, showed good agreement. In the second case, the burst release predictions of the mock drug were then compared to those obtained for Activin A using the same mock-protein optimized particle design. Good agreement between the predicted releases from both protein-carrier systems was achieved with equal formulation characteristics. Also, the predicted release profiles from both proteins match the specified desired release profile closely.

All in all, it was shown that the proposed hybrid model approach could be utilized to aid the optimal design of new drug-carrier systems, based on key molecular descriptors of the drug by manipulating specific formulation characteristics. Also the use of a mock-protein showed to be an important tool to aid the discovery of the working space of acceptable formulation characteristics, given that a good drug representative mock candidate is found.

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Chapter 6 - Conclusions and Future Work

6.1. General conclusions

The general aim of this thesis was to develop a hybrid model based framework for rational controlled drug release design with particular focus on burst release. This framework is composed by 3 key elements:

i) Feature selection to discriminate the key design parameters among a large set of design parameters

ii) Increase predictive power of potentially new designs and Adequate hybrid model structure for CDR systems

iii) Design novel CDR systems to achieve a desired burst release

The detailed conclusions of each element are presented next.

i) Extraction of key input parameters by feature selection

A comprehensive quantitative analysis of the factors (drug molecular descriptors, encapsulation method and formulation characteristics) that impact on the amount of burst release and the burst release rate was made using PLS and decision tree methods. The predictive power of decision tree regression was shown to be significantly better than that of the PLS. Both methods are used to discriminate the input factors which exhibit a significant impact on the amount of burst release and respective kinetics and the findings are largely concordant. Both methods suggest that the amount of drug released during burst is mostly influenced by the formulation characteristics and the synthesis parameters, whereas the drug release kinetics is also influenced by the molecular properties of the drug. The models have been carefully validated, tested and compared with findings reported in the literature. Due to the good agreement of the modeling results with the findings of other researchers as well as the careful validation and testing, the models developed in this part and particularly the decision tree model can be used to design the carrier for a new drug in a way to minimize the burst release. However, it is not possible to predict the cumulative amount of drug released.

ii) Increasing predictive power by a bootstrap aggregated strategy

In the second part, drug release profiles were modeled via the Corrigan equation in which changes in its parameters associated with the total amount of drug released during burst and the burst kinetics are described by Artificial Neural Networks (ANN). The ANNs were derived as functions of the formulation characteristics and molecular descriptors of the drug identified as the most impacting during the decision tree analysis (chapter 3). A bootstrap aggregating identification strategy was used for the development of the hybrid model. After

development, the bootstrap aggregated model was used to predict the cumulative drug release of an independent set of CDR experiments. Good agreement between the predicted and the experimentally measured cumulative drug release profiles was observed.

The aggregated hybrid model was used in a case study, to successfully describe the impact of changes in formulation characteristics on the release profile of ellagic acid.

The overall performance of the developed hybrid model is acceptable and it can be used for optimal drug-carrier design by manipulation of synthesis parameters and formulation characteristics.

iii) Design of a new drug release system

In this part, a PLGA nanoparticle was designed in such a way that a predefined burst release profile was achieved for a α-chymotrypsin. The bootstrap aggregated hybrid model developed in Chapter 4 was exploited in a rational particle design approach that manipulates the formulation characteristics to match a desired release burst profile.

The rational design method was applied to a mock protein (α-chymotrypsin), which exhibits similarities with an expensive protein, Activin A, in terms of important molecular descriptors.

A sensitivity analysis was performed for the impact of the optimal formulation characteristics on the burst release profile and the results, when compared to literature findings, showed good agreement. The burst release predictions of the mock protein were then compared to those obtained for Activin A using the same particle design obtained for the mock-protein. The predicted releases from both protein-carrier systems with equal formulation characteristics were in very good agreement. Also, the predicted release profiles of both proteins match the specified desired release profile closely.

All in all, it was shown that this hybrid model based design framework could be utilized to aid the optimal design of new drug-carrier systems. It was also discussed that the use of a mock-protein can be an important tool to aid the discovery of the working space of acceptable formulation characteristics, given that a good drug representative mock candidate is found.

The results shown in this thesis are only valid for PLGA carrier design, in which the model describes the impact of design choices on the control drug release. The strategies implemented can however be extended to other types of encapsulations. The bootstrap aggregated hybrid model developed was shown to be a powerful tool for predicting the initial burst release when manipulating the formulation characteristics of the particle design to

achieve the desired profile. When compared to classic mechanistic and empirical modeling approaches, which are specific for the drug-carrier pair, hybrid model strategies allow describing and predicting the burst release behavior of a novel drug-carrier system, without carrying out drug release experiments. This knowledge-based strategy is an important step away from the intensive time consuming method of trial and error, during the PLGA particle design. These features of hybrid models have the potential to reduce timelines, save resources and improve drug encapsulation.

6.2. Future Work

It has been seen that the model performance, i.e., the predictions of the cumulative drug release could be improved. Two basic suggestions for improving the model performance in the future are:

i) Improve and extend the categories and classifications that are comprised in the database.

Incorporate more information that better describes the hydrophobicity, permeability and solubility of the drugs by means of the incorporation of their logP and BCS (*Biopharmaceutics Classification System*) classification [1,2]. The information conveyed on the logP of a drug molecule does not suffice by itself to determine how well it would be soluble in a given solvent. Hence the information on where the drug is positioned on the BCS referential would be a useful to aid the choice of the optimal solvents.

ii) One of the challenges of this work was the disparity of the information referring to a release experiment. The lack of original release profile data, led in many cases to extracting this information resorting to the use to image extracting software, with associated uncertainty. Also the particle synthesis manufacturing procedure utilized is referred to by different names, leading to some confusion. Further, it has been seen that the amount of data is a limiting component for the modeling. Furthermore, the addition of more information (as suggested before), in terms of adding more variables to the database, only makes sense when increasing the amount of experiments. Hence, it would be of great interest to create a unified database in the future. For instance, a internet based database could be created where scientists could directly upload the information pertaining to the controlled drug release experiment as well as the release profile, drug information (smile, fasta or pdb file) and formulation characteristics of the reagents and synthesis parameters. When dealing with larger drug molecules (proteins) it was many times challenging to pinpoint the exact amino acid sequence of the entity used for encapsulation, i.e., frequently the researcher would utilize fragments of the protein, or a protein combined with other entities. If this information were to be provided it would improve the predictions of the model, by having more realistically representative molecular descriptors as inputs.

The incorporation of more and also more qualitative data could increase the performance of the model in the future because augmenting the number of variables that could add additional information. Sharing of the experimental data and accurate reporting of the associated

experimental methods in a database could therefore greatly help to exploit the full capabilities of modeling tools.

Model for the entire drug release phase. This work focuses on the initial phase of drug release due to being identified as the most challenging to predict and control. However, any efforts to establish general models for the entire duration of controlled release experiment requires more data and hence the need for a unified database. An attempt to establish an aggregated hybrid model for the prediction of the drug release of the full duration could utilize the "complete" Corrigan equation (equation *8*). This model accurately describes all three phases of drug release that can be observed: initial burst, subsequent near zero-order drug release, and second fast release phase due to polymer erosion.

This work has shown that it is possible to rationally design the particle such that a desired release profile is obtained. This opened the door to tailoring the release profile to the needs of the patient, since e.g., the desired release profiles could vary patient by patient as they have different weight, age, etc. Thus, it seems possible to establish personalized release profiles (personalized medicine). One possibility would be to include patient information as inputs into the model, such as e.g., suggested by Clifton et al. [3].

6.3 References

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