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**DRUG RELEASE FROM PLGA PARTICLES WITH
POLYELECTROLYTE COATING**

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**SCHOOL OF MATERIALS SCIENCE AND
ENGINEERING**

2014

**DRUG RELEASE FROM PLGA PARTICLES WITH
POLYELECTROLYTE COATING**

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A thesis submitted to Nanyang Technological University in
fulfillment of the requirements for the degree of
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“The whole object of travel is not to set foot on foreign land; it is at last to set foot on one’s own country as a foreign land.”

- G.K. Chesterton

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Nomenclature

$\log P$	log of partition coefficient in n-octanol/water
T_g	Glass transition temperature
ACN	Acetonitrile
AFM	Atomic Force Microscopy
ALG	Alginate
ANOVA	Analysis of Variance
BCA	Bicinchoninic acid
BSA	Bovine Serum Albumin
CF	5(6)-Carboxyfluorescein
CHI	Chitosan
CLSM	Confocal Laser Scanning Microscopy
DCM	Dichloromethane
DES	Dextran sulfate sodium salt
DLS	Dynamic Light Scattering
DLVO	Derjaguin and Landau, Verwey and Overbeek
DMSO	Dimethyl sulfoxide
F/P	The mole ratio of FITC to the polyelectrolyte
FDA	Fluorescein diacetate
FITC	Fluorescein isothiocyanate
HPLC	High Performance Liquid Chromatography
IV	Intrinsic viscosity

LBL	Layer-by-layer
MW	Molecular weight
MWCO	Molecular Weight Cut Off
PAA	Poly(acrylic acid)
PAH	Poly(allylamine hydrochloride)
PASA	Poly(L-aspartic acid)
PBS	Phosphate Buffer Saline
PDI	Polydispersity index
PEI	Poly(ethyleneimine)
PEM	Polyelectrolyte multilayer
PGA	Poly(glycolic acid)
PLA	Poly(lactic acid)
PLGA	Poly(D,L-lactic- <i>co</i> -glycolic acid)
PLL	Poly-L-lysine
PSS	Poly(sodium 4-styrenesulfonate)
PVA	Poly(vinyl alcohol)
SEM	Scanning Electron Microscopy
SGF	Simulated Gastric Fluid
TRIS	Tris(hydroxymethyl)aminomethane
UV/Vis	Ultraviolet/Visible

Abstract

PLGA is a traditional encapsulation matrix for drug release due to its biocompatibility and biodegradability, as well as release control manipulation through different physical parameters. Despite the versatility of PLGA, burst release remains a problem. It incurs unnecessary loss of drug, reduces period of sustained delivery, and causes potential overshoot of the drug's therapeutic window.

It is known through the vast literature on PEM microcapsules that PEM film built on drug crystals serve as barrier to the releasing encapsulated drug. It has been applied to, commonly, liposomes, nanogels, and more recently PLGA nanoparticles.

Fundamental studies of the release kinetics on systems with PLGA as the template, however, are lacking, as studies have generally been more focused on demonstration of PEM efficacy in surface functionalization, targeting, and cellular uptake. It is not known, therefore, how much PEM could control the drug release from PLGA.

The objective of this study is therefore to determine the kinetics of drug release when PEM is applied on PLGA. This includes the conditions in which PEM functions as additional diffusional barrier to the drug release, and to determine the factors affecting such release.

We managed to establish PEM on PLGA particles and films encapsulating both low and high MW drugs, with the aim of achieving release retardation. We monitored the drug loss during coating and confirmed a correlation between drug loss and release reduction.

We also optimized the release curve in order to evaluate the release retardation,

since certain shapes of the release curve would not enable analysis of the release retardation. Finally with the evidence of size dependence, we decreased the particle size and highlight the potential role of aggregation in release retardation. A mathematical analytical description was evaluated on the basis of Fickian diffusion, and the role of particle size is further explained.

The novelty of the work stems from the fact that nobody has in detail probed the release kinetics of PLGA-PEM. All arrived at a conclusion that release is reduced, but without any further explanation of why such is the case, and how it relates to other parameters: the PEM built, the PLGA substrate, and the drug studied.

CHAPTER 1

Introduction

1.1 Background

PLGA is a traditional encapsulation matrix for drug release due to its biocompatibility and biodegradability,¹ as well as release control manipulation through different molecular weights, blends, and end-capping.² It can be formed into various dosage-forms: pellets, particles, films, or injectable *in-situ* forming implants,³ encapsulating genetic materials,⁴ vaccines,⁵ proteins,⁶ vitamins⁷ and anti-cancer drugs,⁸ which makes it versatile for many drug delivery applications. To-date, therefore, several commercial products based on PLGA have appeared on the market.⁹

Despite the versatility of PLGA, burst release remains a problem in drug delivery systems such as PLGA particles.¹⁰ It incurs unnecessary loss of drug, reduces the period of sustained delivery, and causes potential overshoot of the drug's therapeutic window. As such, proposed solutions in the literature are many, from surface extraction, modification, coating, to tailored drug distribution in the matrix.¹¹

It is known through the vast literature on PEM microcapsules that PEM film built directly on drug crystals serves as barrier to the releasing encapsulated drug.¹² Thus PEM can be built on PLGA particles to retard drug release, as has been reported by Zhou *et al.*¹³ They noted the effect on the release with different outermost layers and cross-linking of the PEM. It shows the potential of PEM for release control and the modifications that can be done on it.

1.2 Problem statement

In the last decade, many other works on coating of PLGA with polyelectrolytes have been done. Polyelectrolytes such as aminodextran, protamine, PLL, PEI, chitosan, alginate, PAA, and polyelectrolyte pairs such as DNA/PEI, heparin/PLL, and CHI/ALG have been used to coat PLGA particles.^{14–24} It expands the idea of PEM microcapsule by retaining the template, upon which the polyelectrolyte coating is built, rather than dissolution of the template to create hollow microcapsules, as is typically done. However, the reasons for the coating are diverse, and control of drug release is seldom the reason. Most focus on either its mucoadhesive property,^{15,22} surface functionalization,^{18,20} transfection capability,^{21,23,24} or cellular uptake,^{19,25,26} leaving release control only as a side note. The study on release control and therefore, permeability of coatings built on particles, is lacking.

To our knowledge, only PEM coated on nano-sized particles has the drug release retarded, whereas those encapsulating PLGA particles at the micron-sized have not been reported to exhibit drug release retardation. Equally lacking are studies correlating parameters affecting release retardation to the parameters of the drug, particles,

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and PEM. And although the relationship with these parameters could be estimated, there is no study determining the dominant parameters, the list of which is tabulated in Table 1.1.

Table 1.1 – List of possible effects of various parameters on drug release retardation

Parameter	Effect on release retardation	Remarks
↑ Drug MW	↑ retardation	Decreased diffusivity through the PEM
↑ Drug polarity	↑ retardation	Lower partitioning into the PEM
↑ Drug loading	No effect	Diffusion through PEM is not dependent on drug loading
↑ Particle size	–	Depends on whether partitioning or drug diffusivity is the dominant factor
↑ Particle porosity	↓ retardation	Higher open porosity leads to less PEM coverage
PEM related parameters	–	Depends on whether partitioning or drug diffusivity is the dominant factor

1.3 Objective

The objective of this study is therefore to determine the range of release retardation of PEM-coated particles, to understand the factors affecting the release retardation, and to develop a reasonable explanation of such retardation behaviour. The focus therefore, is not to investigate every factor that we have tabulated above, but to attempt to locate the range in which PEM coating effectively retards drug release. Partitioning coefficient might be the dominant factor in effecting the retardation from PEM-coated particles.

With the high number of parameters involved (see Table 1.1), it is highly impractical to create a full-factorial design for such study. The remaining factors that are affected by a change in one factor is also a reason for avoidance of such design. The

heuristic method starts from pivoting of the first immediately recognizable factors to investigation of the next responsible factors. We therefore start our investigation from small MW drug and at micron-sized particles, as opposed to having all drugs, particle sizes, and PEM with a full-factorial experimental designs. This would instead multiply the number of experiments needed greatly.

This study also does not pretend to link between mesoscopic length scale of the polyelectrolyte coating and release retardation. As such, we do not make any consideration on parameters that are typically invoked in physical PEM studies, such as degree of counterion complexation, stoichiometry of the PEM, and degree of interdigitation, which we deem to be “extraneous” to the determination of permeability due to more easily accessible parameters at higher abstraction level.

1.4 Novelty

While the literature contains reported works on PLGA microparticles, to our knowledge, no work reported on the effect of PEM on such particles exists. Although at smaller, nano-sized particles, some works exist that acknowledge the effect of PEM on release retardation, the range and various factors affecting the release kinetic is rarely discussed nor understood. As stated before, works that exist only claim a simple reduction in release with PEM coating.

To our knowledge, this is the first study that reports the relationship between polyelectrolyte coating on PLGA particles and the amount of drug lost during the process, as well as demonstrating the lack of PEM functioning as permeability barrier in larger microparticles compared to smaller nanoparticles. The effect of pH as well as poly-

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electrolyte type on the release retardation from any drug release platforms have also not been reported before, which show that the versatility of PEM build-up extends also to PLGA substrate, similar to build-up on conventional planar substrate such as silicon or glass. We hope, therefore, that this study can provide a basis for PEM coating on PLGA.

CHAPTER 2

Literature Review

2.1 Poly(D,L-lactide-co-glycolide)

Although PLGA can be synthesized in various ways,²⁷ the synthesis is typically done by ring-opening polymerization of cyclic diester lactide and glycolide²⁸ (See Figure 2.1). The lactide/glycolide composition can be varied, leading to varying crystallinity and melting temperature, and at comparable lactide/glycolide ratio, the microstructure becomes amorphous with its own T_g . The most practical composition used for PLGA, however, is the racemic mixture of D,L-PLA (although pure enantiomers exist), due to its being a better film-former; PGA amount is also limited to at most 50% due to difficulty in processing.²⁹

2.1.1 PLGA degradation

PLGA is hydrolyzable into both its acid components, which renders it bioresorbable in the body, since both acids are readily taken up into the metabolic pathways of the

Figure 2.1 – Molecular structure of PLGA. x and y denote the lactic acid and the glycolic acid unit number, respectively. When the acid group is replaced by an ester group, it is ‘end-capped’, and is more hydrophobic than its acid counterpart.

body.¹ It undergoes ‘bulk erosion’, encompassing the whole body of polymer, due to faster water penetration than the polymer backbone degradation, as opposed to ‘surface erosion’³⁰ (see Figure 2.2).

Size, pH, crystallinity, and hydrophilicity play a significant role in determining the rate of degradation.³¹ Both low and high extremes of pH, for instance, contribute to faster degradation.^{32,33} And with increasing size, autocatalysis within the core accelerates the degradation due to carboxyl end groups of degradation products that are unable to diffuse out.^{34,35} Degradation is also faster with increasing amount of glycolic acid in the composition due to increasing hydrophilicity.³⁶ Such effect, however, can be overwhelmed when there is sufficient increase in crystallinity caused by increasing number of glycolic acid unit, which inhibits water penetration.³⁷ These considerations lead to degradation on PLGA microparticles being dependent on the particle size and porosity.³⁸ Porous and smaller particles prevent oligomers from being localized within the core and thus accelerate degradation.

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Figure 2.2 – Schematics of bulk and surface erosion on a sphere. Lighter shades indicate lower MW.

2.1.2 PLGA microspheres

PLGA is commonly used in the form of microspheres due to its ease of administration and versatility in release profile modification.³⁹ There are several commonly employed methods of preparation: solvent evaporation/extraction with single or double emulsion, coacervation, and spray-drying.³ Solvent evaporation/extraction works by oil-in-water emulsion of dissolved PLGA in the oil phase, usually with the aid of surfactants such as PVA.⁴⁰ The solvent is then either extracted with other solvents or simply evaporated (see Figure 2.3). Coacervation is a method done by separation of the oil phase by another organic solvent. The polymer is dissolved containing the drug; another non-solvent is then added into the system while stirring, creating coacervate droplets of the polymer phase. The droplets are further hardened by extraction of the solvent via a larger quantity of non-solvent. Unlike the solvent evaporation method, the absence of the water phase minimizes the drug lost through dissolution in water. Spray-drying, on the other hand, works by spraying the oil phase containing the drug into small droplets. The solvent is immediately evaporated in the spray-dryer and the particles are collected in the collector. In these methods, the drugs may either be co-

dissolved with the polymer or emulsified into the polymer oil phase, creating a double emulsion (W/O/W) in the case of the solvent evaporation method.

Figure 2.3 – Schematics of the solvent evaporation technique

The processing parameters of the solvent evaporation method affecting the drug release can be tweaked, resulting in different effects on the particle morphology, encapsulation efficiency, and size.³⁹ Porosity, for instance, depends on the amount of the water phase in both the first and the second emulsion, in the case of a double emulsion. Reducing the amount of the first water phase reduces the porosity,⁴¹ as well as increasing the second one.⁴² Encapsulation efficiency—defined as the percentage of drug encapsulated against the theoretical amount of drug used during the fabrication—is increased with increasing polymer concentration,⁴³ and both low and high temperature during fabrication.⁴⁴ Size is increased also with increasing polymer concentration^{43,45} and decreasing surfactant concentration,⁴⁶ and the presence of salt in the final water phase is able to change the surface morphology.⁴⁷ In any case the parameters often are interrelated, and changing the particles in turn changes the release characteristics.

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2.1.3 Release and burst release from PLGA

Various schemes for drug release from PLGA matrix has been proposed. In general, however, at least 4 mechanisms can be distinguished: diffusion through water-filled pores, diffusion through the polymer matrix, osmotic pump, and erosion.⁴⁸ The resulting release profile could then take any combination of 3 different phases: burst release, diffusion-controlled release, and a final erosion-controlled release resulting in either a single, biphasic, or triphasic release.

Burst release or phase I is often attributed to un-encapsulated drug on the surface or very near to the surface, for which strategies to solve have been extensively studied,^{10,11} while diffusion-controlled release, phase II, is attributed to either limited porosity at the surface,⁴⁹ drug aggregation,⁵⁰ drug-polymer interaction,⁵¹ or closing of previously open pores.⁵² The final phase III simply occurs due to degradation.⁵³

The drug release characteristics from particles can be influenced by many factors, such as particle size, polymer MW, porosity, temperature, and pH.^{39,54} Since release is either diffusion or degradation-controlled, factors affecting them directly will affect the release. Higher polymer MW, for instance, is able to change the release shape from biphasic to triphasic, with the consequence of delayed release.⁵⁵ Degradation and diffusion-related factors, however, can couple. In simple diffusion-controlled release, for example, smaller particles release faster due to shorter diffusion pathways. Autocatalytic effect however, can reduce this effect or even reverses it, with the effect being more prominent on non-porous particles due to greater autocatalytic effect.^{38,56} Other medium-related factors such as higher temperature results in much faster re-

lease, and due to its variation of T_g with pH, changes of pH are able to induce extreme changes in the release profile, with lower pH exhibiting slower release compared to high pH.⁵⁴

Some mechanistic theoretical models assume that burst is integral of the release. This means burst release is part of the total release described in a typically explicit analytical form that does not distinguish burst from the rest of the release.^{56–62} These models highlight, instead, other aspects such as dissolution,⁵⁹ or degradation,^{57,58,60,61} with the burst taken over implicitly by the dominant release-controlling process, typically with an increasing diffusion coefficient related to matrix degradation, or one controlled by the porosity. As a result, burst release is an afterthought. Others, however, assume a separate source for burst, commonly drug near the surface or located in accessible occlusions or pores.^{63–65} Intuitively, it is agreed that such is the case.⁶⁶

For instance, the model of Batycky *et al.*,⁶³ which was followed by Lisa and co-workers.⁶⁷ In this model, it is assumed that the burst is due to drug attached to the surface. As a result, the equation governing the burst is describing desorption of drugs from the surface. The other component is the diffusion-controlled release, which is ‘active’ only after a certain incubation time. Before the incubation time, therefore, only drug from the surface is able to release, whereas after the onset of mesopore formation, drug from within is also enabled to contribute to the release. Due to its dual-component drug release, the total amount of burst is independent of the drug in the diffusion component. Accordingly, the drug depleted during the coating process should come from the drug attached to the surface, i.e. the burst component, with the drug in the diffusion component left untouched.

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The same holds true with a similar model by Rothstein *et al.*⁶⁵ Although the model employs induction time on a distribution instead of a single value, the underlying cause for burst remains the same. Drug located on the particle radius less than occlusion size (effectively drug on the surface) is released immediately with a maximum effective diffusion, unlike those located within the matrix, where the diffusivity is lowered by a porosity factor. With this paradigm, burst remains essentially separated from the rest of the release, explicitly. A similar mechanism is also at work even with the use of a non-separate burst model, for example by Ehtezazi *et al.*⁶² Although the highlight of the model is the dependence on porosity, practically burst can be interpreted to result from the drug located in accessible pores in the beginning.

Based on these frameworks, therefore, conceptually burst can be compartmentalized into another source, so that treatments affecting this source can be isolated without affecting the rest of the release phases. This is important when discussing the effect of drug loss on the release reduction.

2.2 Polyelectrolyte multilayer

2.2.1 PEM assembly

PEM has been explored greatly at various abstraction levels and for various purposes, ranging from the fundamentals in relation to physical models,⁶⁸ to applications in the biomedical field such as stimuli responsive films,⁶⁹ biomaterial surface modifications,⁷⁰ and composite polymer capsules.⁷¹ This section concerns itself with the aim of presenting the deposit of knowledge in the literature necessary for investigations

in the pursuit of release control.

Figure 2.4 – Examples of common polyelectrolytes and their molecular structure: PAH (a), PLL (b), PSS (c), and DES (d). The origin of the charge of polyelectrolytes is clear.

LBL assembly has several advantages over other thin films developed by the Langmuir Blodgett technique or the self-assembled monolayers, *e.g.*, variety of polyelectrolytes for assembly, stability of film produced, variety of substrate for build-up, and loading capacity of the film.⁷² It works by electrostatic attraction of a polyelectrolyte to an oppositely charged surface. Due to charge overcompensation by the adsorbed polyelectrolyte, the next oppositely charged polyelectrolyte is able to adsorb onto the previous polyelectrolyte layer. Typically a washing routine is performed in-between to remove unbound polyelectrolyte, and the steps can then be continued indefinitely.

PEM build-up is typically monitored by observation of thickness increment or, on capsules, electrophoretic movement of the particles. Very recently there is an inter-

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esting finding that turbidity observation on polyion complexes can also be used to monitor PEM build-up.⁷³ Based on the former, it has been known that PEM build-up is dependent on a number of factors. Number of layers, pH, ionic strength, polyelectrolyte concentration, and charge density can all be tweaked during the build-up process to impart significant change on the morphology and thickness of the resulting film. Jumps in salt concentration⁷⁴ during washing step, as well as sonification,⁷⁵ on the other hand, can be detrimental to the film's stability.

Figure 2.5 – Schematics of the LBL assembly. Figures are not to scale.

During build-up, thickness variation with the number of layer takes either a linear or exponential dependence; the latter due to diffusion of polyelectrolyte in and out of film, with thickness increment proportional to the current thickness.⁷⁶ Which growth regime a PEM falls under depends on the type of polyelectrolyte pair and the complexation enthalpy,⁷⁷ although exponentially growing one can cross over to the linear regime upon reaching a threshold thickness,⁷⁸ and linearly growing one to the exponential regime with increasing ionic strength or temperature.^{79,80}

The type of salt⁸¹ and higher ionic strength are also known to induce thicker film at the same number of layers, with mostly power law dependence^{79,82,83} for the latter. It should be noted that the growth is not always clear-cut. Some reported the presence of a threshold layer below which the structure is distinguishable from the proceeding layers, normally at around 4 layers, and is easily recognizable by—in a linearly growing film—the onset below which the linearity is not followed.^{82,84}

The effect of charge density of the adsorbing chains, meanwhile, falls under 3 regimes.⁸⁵ The first regime exhibits no growth because of low charge density. Above a certain charge density thickness increment decreases as charge density increases because of fewer chains needed to compensate for the opposite charge. Finally there is a saturation regime where thickness increment is constant even at higher charge density. This is caused by strong screening due to counterion condensation. In weak polyelectrolytes, the interplay between pH and charge density due to pH dependence of ionization degree can affect the thickness of the resulting film.^{86–89}

It is known, in addition, that the substrate/underlying layer has an effect on the growth of PEM, resulting in differences in the precursor layers from the multilayer regime.^{82,90} Surface roughness in turn also produces variation in PEM thickness, with PEM produced being less on smooth surface than that on a rough surface.^{91,92}

2.2.2 PEM structure

The typical structure of PEM inside the film does not follow a strictly stratified set of layers, but that of interdigitation, also named the fuzzy layers,⁹³ although exceptions exist.⁹⁴ Each concentration of a polyanion/polycation can be imagined to follow a

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sinusoidal profile, but the sum of concentration of one type is constant along the film normal because of superimposition of sinusoidal concentration profile with the next adjacent layer of the same type.

Some theoretical works were done in the early years of PEM research to explain the conformation upon adsorption,^{95–97} and this has been used to explain some features of the film structure. Basically at low ionic strength, due to low charge screening between chains, polyelectrolyte assumes a flat conformation, in contrast with loops and tails resulting from polyelectrolyte chains assuming an ‘uncharged’ behavior at higher ionic strength. A later experiment observation has supported such evidence.⁸⁴

Ionic strength during build-up also proves to affect the topography, from featureless at low ionic strength to vermicular features at higher ones and increasing number of layers.⁸⁴ These vermicular features are also exhibited by exponentially growing film, in contrast with constantly granular features by linearly growing one.⁷⁶ These features, similar to the above, are also related to the chain conformation in the solution. Chain mobility has also been shown to be affected by ionic strength during build-up, implying that some degree of permeability control is possible.⁹⁸

2.2.3 PEM permeability

Whereas studies on build-up and structure such as shown above are abound, studies on permeability have mostly been done investigating the separation capability of PEM on ions, macromolecules, gas, and solvents,^{99–104} also known as the perm-selectivity of PEM. However, since the goal is to either include or exclude totally the permeation of specific species, their relevance to the permeability aspect of controlled release of

neutral drugs with low molecular weight is lacking. A review¹⁰⁵ on permeability for controlled release does exist, but they focus on the permeability of PEM capsules, not PEM on particles.

Figure 2.6 – Schematics of PEM capsule formation. Number of layer in this instance is 3. Not to scale.

Early studies on permeability focused on planar films; some of which were done with ions¹⁰⁶ or fluorescent agents¹⁰⁷ as permeants. The former utilizes electrochemical properties, which correlates with permeability, and the latter with quantitation of fluorescence intensity indicating permeability. In all and at the same thickness, permeability decreases in the presence of salt during build-up. The explanation offered was that at higher ionic strength, PEM is denser due to less electrostatic repulsion. The alternative is that higher ionic strength gives leeway for defects being “smeared out”.

Later works on capsules gave rise to conflicting result, with some concluding higher permeability with higher ionic strength during incubation (not to be confused with ionic strength during build-up),¹⁰⁸ while others the exact opposite,¹⁰⁹ perhaps due to complication between drug dissolution and ionic strength. But it is known that permeability decreases fastest with the first few layers,¹¹⁰ probably due to permeation

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through pores that are quickly covered by subsequent layers.

Time-scale wise, studies of permeability of PEM capsules encapsulating drug crystals have shown that complete release is inhibited by minutes to hours,^{109–112} that is, in the order of the time scale of drug dissolution, for small molecular weight permeants in the order of $\sim 300 - 400 \text{ g mol}^{-1}$. Works with coating of nanoparticles¹³ encapsulating similarly small molecular permeants, and of liposome¹¹³ encapsulating larger molecular weight permeants, however, suggest that the inhibition can be extended to days. Since permeability has only been determined in works with drug crystals and not with particles, perhaps because of no direct available calculation methods, it is hard to conclude the real determining factor of the time scale, although the relative size of the layer to the core, and the drug molecular weight, are both suspect.

Permeability of the capsules is also further amenable through annealing.¹¹⁴ Permeability reversal with pH is well-known with PAH/PSS. When assembled at pH 7, the capsules are open at low pH and closed at $\text{pH} > 7$, due to mutual repulsion at more charged condition at low pH.¹¹⁵ Calculations on the conformation have shown that the chains are more stretched when it is charged.¹¹⁶

2.3 PEM coating on nanoparticles

It is believed that at sub-micron size range, PLGA particles hold promise for greater tissue and cellular penetration.^{117–120} This has been shown on human colon adenocarcinoma cell line,¹²¹ gastrointestinal tract,¹²² rabbit conjunctival epithelial cells,¹²³ and fungal cells,¹²⁴ where smaller particles have greater uptake than larger ones. Compared to microparticles, the size of the nanoparticles permits various pathways

of endocytosis, either receptor-mediated or not.

It also has wide-ranging applications for cancer treatment, vaccination, inflammatory diseases in the lung, eye, bowel, infections, cerebral and cardiovascular diseases; encapsulating proteins, nucleic acids, vaccines, and small hydrophobic drugs.¹²⁵ As such vast amount of energy in the academic world has been dedicated to providing solutions employing PLGA nanoparticles, as demonstrated by the number of reviews in publication within the last 2 years only.^{8,117,119,125,126}

On particles of such size range or even lower, there have been only a few reports working on coating PLGA nanoparticles with PEM,^{13,127,128} and although works on nanocapsules are extant more than PLGA, those studying the release kinetics are scant. More abundant literature revolves around the use of liposomes^{129,130} or other nanoparticles with PEM coating.¹³¹ Considered soft-matter nanoparticles, these liposomes have been successfully coated with PEM, showing reduction in the release upon coating. A partial list of such systems employing PEM is presented in Table 2.1. Comparison of these systems enables understanding of the performance of PEM on PLGA at the sub-micron level.

As seen in Table 2.1, PLGA nanoparticles coated with CHI alone was reported to show insulin release reduction.¹²⁷ The CHI layer, however, was not coated after PLGA fabrication. Rather, CHI was incorporated into the external PVA solution during PLGA emulsion, resulting in a pre-coating of CHI on PLGA. As such, the difference in release behaviour could be caused by other factors at work during fabrication. The presence of CHI could affect the viscosity of the water phase, solvent evaporation rate, and osmotic difference between outer and inner water phase, all of which affect the

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Table 2.1 – Works on nanoparticles coated with PEM for drug delivery

Type	Size (nm)	Drug	PEM	Medium	pH	T (°C)	Release characteristics
PG	~120 – 130	Insulin	CHI	PBS	7.4	37	reduced from ~ 30% to ~ 20% at 2.5 h ¹²⁷
PG	~ 350	Rhodamine 6G	PEI/PAA	PBS	7.4	37	reduced from ~ 30% to ~ 18% at 30 days ¹³
PG	~270	FITC-dextran	PAH/PSS, PLL/DES	PBS	7.4	37	reduced from ~ 90% to ~ 70% at 100 h ¹²⁸
LP	~330	Amoxicillin, Metronidazole	PAH/PAA	SGF	1.2	37	reduced from 100% at 6 h to ~60% at 1 day ¹³²
LP	~250 – 520	Doxorubicin	PAH/PAA	PBS	7.4	37	reduced from ~ 60% to ~ 35% at 12 h ¹³³
LP	~110 – 180	Carboxy-fluorescein	PLL	TRIS	7.4	25, 54	increased for both aggregated and singly-covered PLL samples ¹³⁴
LP	~100	Pyranine	PLL/PASA	HEPES	7.4	25	reduced to almost negligible amount ¹³⁵
LP	~110 – 230	Paclitaxel	PAH/PAA	Phosphate + Tween [®] 80 water	7.4	37	lagging by 2 h, reached 80% in ~2 h ¹³⁶
LP	~200 – 350	BSA	CHI/ALG	water	N.A.	37	reduced from 100% to 50% at 20 days ¹¹³
NG	~50	Procaine HCl	PAH/PSS	10 mM NaCl	7.4	37	reduced from 100% to 45% at 3 h ¹³⁷
NC	~180 – 210	Acridine HCl	CHI/ALG	Phosphate	7.4	25	complete release in 5 h ¹³⁸
NC	~170 – 220	BSA	CHI/DES	HCl, PBS	1.4, 7.4	37	slower release at pH 1.4, complete release in ~5 h ¹³⁹

LP: liposomes, NG: nanogels, NC: nanocapsules, PG: PLGA, N.A.: not available

final particle morphology.⁴⁰

Another model drug, rhodamine 6G (479 Da), has been reported to be encapsulated in PLGA nanoparticles coated with PEI/PAA.¹³ Interestingly, the release is not significantly different in relation to layer number, but only to its outer-most polyelectrolyte: faster when outer layer is positive, slower otherwise. In this case then, the release is modulated not as much by diffusion, but by electrostatic interaction, since it is positively charged. Compared to another work in our group employing FITC-dextran (~70 kDa) with PAH/PSS and PLL/DES coating,¹²⁸ drug release is lowered with layer number, indicating that it is diffusion-controlled. Docetaxel is also similarly retarded by PEI coating from a burst of 1 day to sustained release up to 1 week.¹⁴⁰

Most of the drugs employed by the liposomes are small drugs (< 1000 Da), with the reduction in release shown to be in order of hours, excepting BSA. Unfortunately the buffers used for liposomes are mostly not PBS, the typical buffer used for PLGA in common release studies. Noting that PEM permeability is determined also by its ionic strength,¹⁰⁸ comparison between them cannot be a definite quantitative one. Deduction on the performance of PEM on PLGA from liposomes is also complicated by the fact that liposomes are soft-matter materials composed of potentially re-arranging lipids. In one case, for instance, the release increases upon coating with PLL.¹³⁴ This also explains the relatively low temperature used in some of the release studies (25 °C), due to the 'leakiness' of the liposomes.

It is interesting that throughout the literature on PEM-on-nanoparticles, the mechanism for barrier release is typically only slightly touched, with no elaborate expla-

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nation for the dominant factors for retardation. There has been no attempt to explain the dominant mechanism in terms of partitioning, nor is there any investigation on the factors tabulated in Chapter 1, which, recapitulated briefly, consist of drug-related factors such as MW, polarity, particle-related factors such as size, porosity, and PEM-related factors such as thickness. Works mentioning such factors, such as the odd-even effect of the layer number, exists (see Zhou *et al*¹³), but such works are few and far between. Most works are satisfied with encapsulation or longer sustained release due to PEM coating,^{135,137,139} or with focus on other properties such as bioadhesivity,¹²⁷ treatment of bacterial infection,¹³² tumor,^{133,136} and interaction between the core particle and the PEM.¹³⁴

2.4 Conclusion

Although individually, each PLGA and PEM has been extensively studied for release, structure, and permeability, the study on PEM-on-PLGA itself is lacking. Kinetics studies are focused on PEM capsules, whereas studies on PEM-coated PLGA or nanoparticles are focused on PEM properties other than drug release kinetics.

Noticeably absent is the mention of controlling mechanism on PEM-coated particles, even as Fickian diffusion is virtually acknowledged. It is known, for instance, that additional barrier such as PEM coating could control the release through either the partitioning coefficient or the diffusivity. The lack of mention of such phenomenon is what prompts the study in this work.

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CHAPTER 3

General Experimental Procedures

3.1 Materials

The polymers used throughout the study are indicated by their composition and IV. 'PLGA 75/25' indicates a lactide/glycolide composition of 75%/25% (mol), respectively, and 'PLGA 50/50' a composition of 50%/50% (mol). PLGA 75/25 was purchased from Purac with IV of 1.01 dL g^{-1} , whereas PLGA 50/50 consists of two sets with different IV: 1.18 dL g^{-1} from Bioinvigor and 1.01 dL g^{-1} from Purac. Whenever mentioned, the IV of the polymers is indicated to distinguish the two.

Two types of PVA were used to create particles: for microparticles, 'PVA-403' purchased from Kuraray was used. It had a hydrolysis rate of 78% – 82% according to the manufacturer's specification with MW $\sim 15\,000 \text{ Da}$; for most of the nanoparticles, 'PVA 30000', purchased from Sigma-Aldrich was used, with a hydrolysis rate of 87% – 90% and MW of $30\,000 - 70\,000 \text{ Da}$.

For model drugs, fluorescein, ibuprofen, BSA, and FITC-dextran of different MW

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(3000 – 5000 Da, 70 kDa, and 250 kDa) were purchased from Sigma-Aldrich, as well as FITC for PAH labeling.

The polyelectrolytes used throughout the study were PEI ($M_w \sim 25\,000$ Da, $M_n \sim 10\,000$ Da), PAH (MW $\sim 56\,000$ Da), PSS (MW $\sim 70\,000$ Da), PLL hydrobromide (MW 30 000 – 70 000 Da), and DES (MW 36 000 – 50 000 Da), all of which were purchased from Sigma-Aldrich.

HPLC-grade DCM and DMSO were purchased from Tedia, with Millipore water being used for all the experiments. All salts for buffer preparations—PBS and carbonate buffer—were of analytical grade, purchased from Sigma, except NaCl and KCl, from VWR, and Na_2HPO_4 , from Alfa Aesar.

3.2 Procedures

PLGA particle and film fabrication

PLGA microparticles and nanoparticles were fabricated similarly using either the O/W or the W/O/W emulsion followed by evaporation of the solvent in the oil phase, as is amply available in the literature.^{141–146} Due to its variety, the exact details of fabrication of each type of particles are described in the corresponding chapter. Suffice it to say that the primary difference between micro- and nanoparticles is the emulsion power of the final emulsion: greater with nanoparticles, and smaller with microparticles. The former was achieved here using ultrasonication. After harvesting, all particles were washed by repeated centrifugation and re-dispersion into water before being freeze-dried, except for nanoparticles, which were not freeze-dried but were immedi-

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ately subject to further procedures.

The nomenclature used for the parameters are as follows. O indicates the oil phase, W1/W/W2 the water phase. Emulsion I refers to the emulsion process of W1 into O, and Emulsion II to that of O into W2. Where no roman number is indicated it refers to a simple O-into-W emulsion.

PLGA films encapsulating FITC-dextran were made by the solution casting method. Weighed amount of 1 g of PLGA 7525 was dissolved in 4 mL of DCM with the addition of 0.4 mL of 2.5 mg mL⁻¹ of FITC-dextran. A thickness-adjustable casting knife was used with the film applicator at 50 µm thickness, yielding ~50 µm dry thickness after drying at 55 °C in vacuum for 1 week. Films were either cut into squares of 1 × 1 cm² or circles with Ø2 cm.

Model drug quantitation

FITC-dextran and fluorescein were quantitated by fluorescence spectroscopy, with 490 nm/520 nm excitation/emission wavelengths for the former, and 494 nm/ 521 nm excitation/emission wavelengths for the latter; ibuprofen was quantitated by UV/Vis spectroscopy at either 222 nm or 264 nm; and BSA was quantitated using the Micro BCA protein assay kit from Thermo Scientific with subsequent absorbance detection at 562 nm. Both fluorescence spectroscopy and absorbance detection for BSA were done on Infinite[®] M200 (Tecan), whereas that for ibuprofen was done on UV-1700 (Shimadzu). In all measurements the calibration curves were made with the corresponding solvents.

***In-vitro* model drug release**

Particles were dispersed into microtubes containing 1 mL of PBS (pH 7.4, and in some cases with 0.01% Tween[®] 20) or carbonate buffer (pH 9.6), and incubated at 37 °C with a horizontal orbital shaker shaking at 50 rpm. At specific time points particles were separated from the supernatant by centrifugation, and aliquots of the supernatant were taken for quantitation specific to each model drug. The same amount of buffer was replenished to its original volume. The amount quantitated was calculated for cumulative amount of model drug released from the particles.

Drug loading determination

The content of model drugs in the particles was liberated by either digestion, dissolution, or extraction. Digestion of the particles was done by dispersion into 1 M of NaOH and incubation at 37 °C for either 3 h for fluorescein-containing particles or overnight for BSA-containing particles. Dissolution was done for ibuprofen-containing particles by ACN, whereas extraction was done for FITC-dextran containing particles by dissolution into DCM and subsequent partitioning into PBS by vigorous shaking and incubation at 37 °C overnight under 50 rpm orbital shaking.

Once dissolved/digested/extracted, quantitation of the model drug content was done according to each drug's quantitation protocol. For BSA, an additional step of pH adjustment to ~7 was done prior to quantitation. The drug loading then is defined as follows.

$$\text{Drug loading (\%)} \equiv \frac{\text{Drug amount}}{\text{Polymer amount}} \times 100\% \quad (3.1)$$

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PEM build-up on particles and films

Polyelectrolytes were dissolved in 0.5 M NaCl with a concentration of 1 mg mL⁻¹. For microparticles, the particles were suspended in the polyelectrolyte solution for 15 min and washed with either water or 0.5 M NaCl twice before the next layer build-up of the oppositely charged polyelectrolyte.

For particles with layer number > 30, particle size was increased in order to enable rapid coating procedures. Coating was done at pH 7 in with the polyelectrolyte solutions with 5 min of incubation per layer, and twice washing with 0.5 M NaCl solution at the same pH. After each 30 and 70 layers, the particles were suspended in the same NaCl solution at 4 °C before subsequent coating.

For nanoparticles, particles were re-dispersed in 0.5 mL of water first before addition of 0.5 mL of polyelectrolyte solution due to difficulty in re-dispersion in high ionic strength solution. The coating time was adjusted to 5 min per layer. The pH of the polyelectrolyte solution is indicated whenever adjusted. The washing in-between each coating for nanoparticles was done once in pH-adjusted/un-adjusted water. For particles re-dispersed with sonication, sonication was done for 10 min in an ice bath to prevent temperature increase.

For films, manual dipping procedure was followed with prior cleansing in 0.3% (w/v) PVA-403 and 0.1 M HCl for glass substrate. The same solution concentrations as that used on microparticles were used, with dipping for 15 min in each polyelectrolyte solution and rinsing with water twice in-between. For films, to increase the charge,

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the PEM was coated with PEI as a precursor layer in order to increase the charge, since PEI is a strong polyelectrolyte.

All microparticles were subject to freeze-drying after coating, while nanoparticles were subject to other immediate experimental procedures, and films to vacuum-drying at 25 °C.

Confirmation of PEM build-up

For particles, layer build-up was monitored after every one layer prior to a single washing with water. Laser Doppler Micro-electrophoresis for ζ -potential measurements were done with the Malvern Zetasizer Nano ZS (Malvern Instruments) at 25 °C. ζ -potential was measured in folded capillary cells with water as dispersant, calculated using the Henry equation with Smoluchowski approximation. The sign reversal of the ζ -potential value indicates PEM build-up.

Although sign reversal could indicate, instead, exchange of polyelectrolyte instead of build-up, PLGA itself is negatively charged, whereas PAH positive and PSS negative. For an exchange to take place, PAH must overcome the electrostatic attraction from PLGA, after which PSS must adsorb on the negatively charged PLGA, overcoming repulsion from PLGA. The first step is only possible when the pH is close to the pKa of the polyelectrolyte (at pH 10, for instance) or at even higher ionic strength, thereby reducing the effective charge and enabling other types of interactions to occur. The second step, however, is difficult to achieve without any additional type of interaction. On the investigated polyelectrolytes, there has not been reported such exchange mechanism in the literature.

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Monitoring of the build-up on glass slides was done by advancing contact angle measurement, as was done by Yoo *et al.*,⁸⁶ because preliminary experiments with static contact angle measurement did not show conclusive evidence of successful build-up. The captive needle technique was used to measure the contact angle on both coated and uncoated glass substrate with a water droplet volume of $\sim 2 - 3 \mu\text{L}$. In this technique, the needle was kept in contact with the bubble without dropping it, preventing kinetic energy from distorting the bubble shape. The captured droplet image was then numerically fitted into sphere and the advancing contact angle calculated from the fitted value.

The PEM thickness on glass substrate was gauged by scrapping the film with a razor blade and measuring the vertical difference between the exposed glass surface and the coated PEM using the AFM, following similarly reported procedures.^{84,147}

To visualize the coating layer, CLSM was employed with TCS SP8 from Leica Microsystems. Coated films and particles were terminated with a layer of PSS, on top of which FITC-labeled PAH (PAH-FITC) was coated (labeling procedure following). The presence of fluorescence would indicate successful multilayer build-up of the previous layers.

Microparticles for which ζ -potential measurement was unable to be done was confirmed for coating by dissolution of the core by ACN. Intact microcapsules would prove that the coating was successful. For this purpose optical microscopy for microcapsule imaging in dispersion was done with Eclipse ME600 from Nikon Instruments.

Estimation of the thickness of PEM was done on PEM built on glass substrate. Manual dipping procedures were followed with prior cleansing of the substrate in

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0.3 % (w/v) PVA and 0.1 M HCl. The surface was scratched and the difference in height was measured by AFM (Digital Instruments Nanoscope[®] Scanning Probe Microscope) in tapping mode with silicon nitride tip.

Polyelectrolyte labeling

For PEM coating visualization labeling was done with some modification from the literature.¹⁴⁸ Briefly, 10 mL of 10 mg mL⁻¹ PAH in 0.1 M NaHCO₃ was mixed with 0.5 mL of 1 mg mL⁻¹ FITC in DMSO and dialyzed with cellulose ester dialysis membrane (MWCO 810 kDa) against pure water to remove unreacted FITC. Freeze-dried PAH-FITC was taken and weighed (64% yield), and its absorbance in H₂O was measured at 494 nm and 280 nm. The F/P ratio was calculated to be 1.6, labeled at every 369th position. The absorption spectrum was found to be similar to free FITC.

Morphology and particle size

Particle and film morphology was characterized with JSM-6360 and JSM-6340F SEM (both from JEOL). Microparticles and films were coated with gold or platinum before mounting on carbon tape before imaging. The size of micron-sized particles was measured by imaging through SEM and analysis on ImageJ with a minimum of 500 particles using area-equivalent diameter.

DLS for z-average hydrodynamic radius and PDI measurements were done on Zetasizer Nano ZS (Malvern Instruments). The size distribution was calculated using a built-in auto-correlation function with a backscatter detector at 173°. Size progression during coating of nanoparticles was measured in PBS for correlation with the

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respective release curves, unless otherwise indicated. For microparticles, the dispersant was water.

Statistical analysis

Release studies, loading, size, and ζ -potential measurements were done in triplicate. Statistical analysis to compare significant difference between curves were done with GraphPad Prism™ using two-way ANOVA with repeated measures. To compare two values, Student's t-test was done. Significant difference is confirmed at $p < 0.05$.

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CHAPTER 4

Establishing Polyelectrolyte Multilayer

The emulsion-evaporation process used to fabricate PLGA microparticles employ PVA as a surfactant. It might be suggested that the presence of surfactant on the particle surface is a barrier to PEM formation. It is after all known that a fraction of PVA will always remain on the surface regardless of the number of washing.¹⁴⁹ But our group has previously demonstrated that PLGA assembly is still possible,¹²⁸ since the surface charge remains negative (see Figure 4.1). For both all types of particles, the ζ -potential value of the bare particle is ~ -15 mV. Without PVA, bare PLGA particles are known to reach a surface charge of -45 mV.¹⁵⁰

For nanoparticles, however, due to the different coating conditions, the ζ -potential evolution (the change in zeta-potential with the number of layer) is different. The alternating signs, however, are still observed, although in some cases it is subdued to near neutral charge. The next layer however, re-orient its charge. Furthermore, unlike microparticles, agglomeration was observed throughout the coating on nanoparticles. This is discussed further in Chapter 6. It is notable that the charge from PLL/DES

coating is lower than PAH/PSS, due to the lower charge density.

Figure 4.1 – A typical ζ -potential evolution during coating on microparticles with PAH/PSS (square) and nanoparticles coated with PAH/PSS (circle) and with PLL/DES (triangle)

For particles coated at high layer number ≥ 30 , the success of particle coating was demonstrated by dissolution of the core. Unlike particles coated at 4 layers, capsules formed at 30 layers are robust enough to endure the stress during dissolution of PLGA core. The result of this procedure is presented in Figure 5.5.

The coated PEM on films was visually confirmed by coating an additional PAH-FITC on top of the built-up PEM. Coating of PAH-FITC on successfully built PEM gave rise to fluorescence intensity detectable by CLSM. As a control, PAH-FITC was also coated on top of the similarly positively charged PEI. Fluorescence microscopy revealed lack of fluorescence from such coating (see Figure 4.2), which shows that fluorescence also appears with attachment on previously built layer of the opposite

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charge.

The fabricated PLGA films were of 50 μm thickness. They exhibit valleys on the surface (see Figure 4.2). This surface is the one exposed to the air during drying after film application. The other surface is smooth. The troughs most likely formed during rapid evaporation of the solvent from the surface. Through CLSM of the PAH-FITC-coated film, the depth of the through was found to be in the order of 1 μm .

Figure 4.2 – Visualization of PEM on PLGA films (C) and particles (D) coated with 2 layers before additional PAH-FITC. As a negative control, a piece of PLGA film coated with PEI was immersed in PAH-FITC, showing the lack of fluorescence due to strong electrostatic repulsion from PEI (B). The bright-field image (A) and SEM image (E) of the films are shown for comparison

Even with such geometry, the films are fluorescent after coating. Note that the image is a superposition of two different z-positions. The fluorescence of both in-

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indicate that the coating covers both the peaks and the troughs of the film, although in-homogeneity is found. This technique unfortunately, is only able to confirm the presence of PEM without further qualification. The particles were also successfully coated by confirmation with CLSM. The images here are of 2 layers; at higher layer number both particles and films also exhibit the presence of fluorescence.

Figure 4.3 – AFM images of PEM built on glass substrate at 6 layers.

The thickness of the PEM was estimated by AFM on scrapped PEM on glass substrate with a razor blade (see Figure 4.3), although it should be noted that PEM growth

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could be affected by the substrate.^{82,90} The point of the experiment was to provide an estimate, although not exactly, of the order of magnitude of the PEM thickness. Thus far there is not yet reliable means for measuring directly the thickness of PEM on colloidal particles. The most direct is the measurement of particle size, with increasing size indicative of increasing thickness. But this works only at very small particle size and at very monodisperse particles. The topography of such build-up is shown in Figure 4.3. By scratching, the thickness was found to be ~ 2 nm/layer.

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CHAPTER 5

Polyelectrolyte Coating on Microparticles

5.1 Introduction

As we have mentioned in Chapter 1, there is a lack of literature on drug release from PEM-PLGA microparticles. At the initial stages, therefore, we could only surmise that the behaviour of PEM on PLGA is the same as that on capsules, which have been more often studied. In order to investigate the release characteristic changes with drug molecular weight and drug polarity, small model drugs with varying hydrophobicity are used (listed in Table 5.1), as well as FITC-dextran of various molecular weights and BSA.

The heuristic methodology was started from small drugs with low layer number, as capsules have been created with such parameters. The factor to be considered first is drug polarity. Fluorescein is used as a model hydrophilic drug, whereas ibuprofen

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Table 5.1 – List of low molecular weight drugs encapsulated in PLGA microparticles used in this section

Drug	Chemical structure	MW (Da)	log <i>P</i>
Fluorescein		332	-2.4, 1.8 ¹⁵¹
Ibuprofen		206	3.5 ¹⁵²
FDA		416	4.2 ¹⁵¹

5. POLYELECTROLYTE COATING ON MICROPARTICLES

is used as a model hydrophobic drug. We compare hydrophilicity and hydrophobicity of the drug to see whether there is any effect of hydrophilicity on the release retardation due to PEM. They were selected due to the fact that the encapsulation of ibuprofen and fluorescein sodium—the sodium salt of fluorescein—into PEM capsules have been well known by PEM build-up at pH values at their lowest solubility.^{108–110,153} The release time-scale of these small molecules from PEM capsules is typically in the order of minutes. Since PEM permeability of both fluorescein and ibuprofen has been reported several times in the literature, we incorporated these molecules in PLGA as a comparison, to see whether there is a difference in PEM permeability when PEM does not form as a capsule but as a shell.

5.2 Experimental procedures

Low molecular weight drugs

For fluorescein- and FDA-loaded microparticles, the following parameters were used: O: 0.2 mL of 12.5 mg mL⁻¹ fluorescein in DMSO or 2.5 mg of FDA and 100 mg of PLGA 75/25 in 2 mL of DCM, w: 8 mL of 0.3% (w/v) PVA-403, Emulsion: 2 h at 600 rpm with 20 mm bar, Evaporation: 3 h with addition of 46 mL of PVA. For larger microparticles encapsulating fluorescein, PLGA and the solution volume were scaled 8×, with the rest of the parameters retained.

For ibuprofen-loaded microparticles, the following parameters were used instead: O: 40 mg of ibuprofen in 400 mg of PLGA 75/25 in 2 mL of DCM, w: 20 mL of 0.3% (w/v) PVA-403, Emulsion: 2 h at 1000 rpm with 20 mm bar, Evaporation: 3 h with

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addition of PVA totaling 200 mL.

Microparticles encapsulating ibuprofen for coating at > 30 layers used the following parameters. O: 40 mg of ibuprofen in 800 mg mL⁻¹ of PLGA 50/50 (IV 1.01 dL g⁻¹) in 8 mL of DCM, W: 64 mL of 0.3% (w/v) PVA-403, Emulsion: 2 h at 900 rpm with 20 mm bar, Evaporation: 5 h with addition of PVA totaling 4 L.

High molecular weight drugs

For FITC-dextran containing particles, W1: 1.6 mL of 12.5 mg mL⁻¹ FITC-dextran (of either MW) in water, O: 800 mg of PLGA 75/25 in 16 mL of DCM, W2: 64 mL of 0.3% (w/v) PVA-403, Emulsion I: sonication at 40% for 2 min at 2 mL of O each, Emulsion II: stirring at 600 rpm for 2 h with 20 mm bar for large particles or at 1000 rpm with 40 mm bar for small particles, Evaporation: 3 h with addition of 368 mL of PVA.

For BSA containing particles, W1: 0.2 mL of 120 mg mL⁻¹ BSA in PBS, O: 400 mg (for 160 µm particles) or 200 mg (for 60 µm particles) of PLGA 50/50 (IV 1.01 dL g⁻¹) in 4 mL of DCM, W2: 50 mL of 0.3% (w/v) PVA-403 and 5% NaCl, Emulsion I: sonication at 40% for 2 min, Emulsion II: stirring at 900 rpm for 2 h with 20 mm bar, Evaporation: 3 h with addition of PVA totaling 2 L.

For particles of 2 µm size, W1: 0.2 mL of 120 mg mL⁻¹ BSA in PBS, O: 200 mg of PLGA 50/50 (IV 1.01 dL g⁻¹) in 4 mL of DCM, W2: 60 mL of 0.3% (w/v) PVA-403 and 5% NaCl, Emulsion I: sonication at 40% for 2 min, Emulsion II: stirring at 900 rpm for 2 h with 40 mm bar, Evaporation: overnight with addition of PVA totaling 80 mL.

For porous particles containing BSA, W1: 0.4 mL of 60 mg mL⁻¹ BSA in water, O: 400 mg of PLGA 50/50 (IV 1.01 dL g⁻¹) in 4 mL of DCM, W2: 64 mL of 0.3% (w/v)

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PVA-403, Emulsion I: sonication at 40% for 2 min, Emulsion II: stirring at 700 rpm for 2 h with 20 mm bar, Evaporation: 3 h with addition of PVA totaling 4 L.

5.3 Low molecular weight drugs

PLGA particles loaded with fluorescein and ibuprofen have a loading value of 0.35% and 5.3%, respectively^{*}. The low loading amount of fluorescein was caused by the use of single emulsion method instead of the double emulsion for particle fabrication. Ibuprofen, on the other hand, was encapsulated in greater amount due to its hydrophobicity. During emulsion, drugs are able to dissolve/partition into the PVA phase from the oil phase.^{154,155} With higher hydrophobicity drugs are prevented from such partitioning, yielding better encapsulation.

Microparticles encapsulating ibuprofen virtually exhibits wider particle size distribution than fluorescein. Both particles have virtually no pores on the surface, as seen through the SEI (Fig 5.1), with a size in the order of 10 μm . This rules out the possibility that certain parts of the particle surface is inaccessible to the polyelectrolyte during the coating process.

The drug release for fluorescein is presented in Figure 5.2, with no burst in the beginning up to $\sim 60\%$ in 15 days. Burst release from similarly hydrophilic molecule of similar size such as propranolol HCl is known to be as high as 50%.¹⁵⁶ The fact that it has considerably little to no burst in the beginning is likely due to the low loading value. We have created larger fluorescein-encapsulating particles of the order of 50 μm that shows the same behaviour. At a much lower loading of $\sim 0.1\%$, the

^{*}through protocols described in the Drug Loading Measurement section in the General Experimental Procedures chapter

(a)

(b)

Figure 5.1 – Particle morphology of fluorescein- (a) and ibuprofen-loaded microparticles (b). The morphology of those coated with PEM is the same, *i.e.*, PEM on the particles cannot be visually observed through SEM.

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release is more sustained and linear (Figure 5.4).

Figure 5.2 – Fluorescein release from PLGA microparticles. The release of uncoated and coated particles are not significantly different.

Ibuprofen is similarly released without any burst. This is similar to other reported works with similar drug content, where it easily fits the analytical Fickian diffusion curve from spheres very well.^{156–158} In these works, however, PEM is not employed at all. The difference in loading between ibuprofen and fluorescein should not warrant an invalid comparison, since retardation by the PEM acts at any loading, as far as diffusion is concerned.

The release from particles coated with PAH/PSS up to 5 layers shows that neither fluorescein (Fig. 5.2) nor ibuprofen (Fig. 5.3) was retarded by the PEM layer. The difference between drug release from coated and uncoated particles is insignificant. This extends to additional build up to the 8th layer for ibuprofen, whereas for fluores-

Figure 5.3 – Ibuprofen release from PLGA microparticles. Error bars are smaller than symbols.

cein the same number of layer actually exhibits slightly higher release with significant difference after the same period of time. The latter, however, is attributed to higher wettability. Fluorescein released from larger particle size is also similarly released, without any significant difference due to PEM. Ibuprofen encapsulated in larger particle size, on the other hand, is discussed in the next section.

For comparison, several observations could be made on their microcapsule counterpart. For ibuprofen,^{109,153} the release even at 5 layers of PAH/PSS, twice as many number of layers in our case, is only sufficient to retard the release to 2 min. Fluorescein sodium is also released from^{108,110} and diffused into the microparticles¹¹⁴ in similar time-scale. The latter is reportedly similar in both MW and $\log P$, being only a sodium salt of fluorescein.¹⁵⁹ This time-scale indicates that free-standing PEM

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capsules are permeable to such molecules.

In order to ascertain whether this is caused by low hydrophobicity, FDA was encapsulated into microparticles. FDA has a higher MW and a higher log *P* than ibuprofen (see Table 5.1). The release curve, however, also shows that PEM is not retarding at all. Note that FDA has been used to mimic the release of paclitaxel.¹⁶⁰ Presumably, the release kinetics through PEM would be similar to that of FDA. Since paclitaxel has been reported to experience release retardation with PEM application on liposomes,¹³⁶ whereas from our experiments FDA on PLGA shows no such retardation, this indicates that FDA is not a proper substitute for paclitaxel, or that PEM permeability on liposomes and PLGA is not comparable.

That neither hydrophilic nor hydrophobic drug was able to be influenced by PEM could be caused by the low layer number (< 10). For this purpose microparticles of up to 100 layers were produced. The particle size was increased in order to enable rapid coating procedure. Due to the time-scale needed to produce such layer number, a control sample was provided with simple dispersion in 0.5 M of NaCl at pH 7*.

The control was done in order to show the release curve change simply through immersion in an electrolyte solution. This is the same solution as the solution used for coating but in the absence of polyelectrolyte. Should a change in release curve occur in polyelectrolyte-coated particles, it needs to be shown that such change occurred not due to simple immersion but due to PEM acting as diffusional barrier. This is the

*With the currently used technique, to produce 100 layers needs whole days of coating, since each coating needs at least several minutes for each adsorbing polyelectrolyte to re-arrange themselves in the PEM. This is different from coating on glass slides, where robots have been reported for use in coating up to hundreds automatically. A recent attempt at scaling up this capsule fabrication process has been reported by Caruso's group by immobilizing the particle templates within a gel, enabling the use of robots.¹⁶¹

(a)

(b)

Figure 5.4 – Fluorescein release from PLGA microparticles of $\sim 40\mu\text{m}$ (b) and its particle morphology(a). The release is more sustained compared to particles of lower size, but with the same PEM treatment effect of equal release to uncoated particles.

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purpose of the control sample. For the same reason ibuprofen (which is hydrophobic) was chosen to minimize drug loss during the coating process.

Based on the SEI, it is apparent that the size of the particles is in the range of 50–200 μm . PAH/PSS multilayer is rarely assembled at high layer number on particles; it has however, been commonly assembled up to 100 on planar surfaces*.

Because of gravitational force on particles within this size range, it is not possible to use laser-scattering methods to detect its mobility for the calculation of ζ -potential. We have, instead, qualitatively confirmed the success of PEM formation by dissolving the PLGA template by ACN. Based on Figures 5.5c–5.5f, it can be seen that intact capsules are discernible. The ‘buried’ appearance of the capsules in the SEI was caused by PLGA, since PLGA was unable to be completely removed from the capsule surface even with > 10 repeated washings in ACN. To ascertain that these were the capsules after core dissolution, optical micrographs were taken with the capsules dispersed in water. In all circumstances no ibuprofen crystal is visible, indicating complete removal of PLGA along with ibuprofen with the dissolution process (noting as well that ibuprofen is many times smaller than PLGA chains and equally soluble in ACN).

The bare particles themselves have smooth surface morphology (see Figure 5.5a), with no noticeable pore, either open or closed. With such surface and at 100 layers of coating, the coverage should be total, as indicated by the intact capsule formation. The coated particles at 100 layers are virtually indistinguishable from its bare counterpart (Figure 5.5b). It is to be noted that at lower layer number of 4, we were unable to form *intact* capsules by dissolution. PEM films were discernible after dissolution but did

*Number of layers in the literature vary from 10,^{162–164} 18¹¹⁰ layers on particles and 20,¹⁰⁶ 25,¹⁶⁵ 30,⁸⁰ 80¹⁶⁶ on planar substrates

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not form closed-formed capsules such as seen in the figures. Rather, it takes the form of loose broken patches of films (Figure 5.6).*

This shows that microcapsules with higher layer number are more resistant to environmental stress from the dissolution process. It is also noteworthy that to-date, according to our knowledge, no PEM capsules built on PLGA substrate have been reported. The intact capsules resulting from PLGA template are unsurprisingly similar in appearance to capsules produced with the commonly used CaCO_3 template in the literature.^{92†}

The PEM at higher layer number is different from those at lower layer number. The first few five or six layers, dubbed the ‘precursor layer’ in some literature, are affected by the substrate and exhibit heterogeneity in the form of holes.^{82,90} In the precursor layer zone (up to 6 layers), the PEM morphology does not depend on whether the number of layer is odd or even, unlike the bulk layer (above 6 layers) where the features are regulated by the odd/even number of layers, *i.e.*, 10 layers similar to 12, 14, 16, *etc.*, and 11 layers similar to 13, 15, 17, *etc.*

As the number of layers increases and goes beyond the precursor layer, the PEM enters zone II, where growth is regular, cyclical (depending on the odd/even number of layers), and homogenous. Dependence on the substrate and the precursor layer, therefore, is very low. There should be no ‘detachment’ of subsequent layers due to high layer number, since the electrostatic force is orders of magnitude higher than

*To obtain capsules, PEM-coated particles were dispersed in acetone to dissolve the PLGA core, supposedly leaving the PEM shell intact. They were then centrifuged, and re-dispersed again in the acetone. This procedure was repeated several times to remove the remaining PLGA. With particles coated with 100 layers of PEM, capsules were immediately obtained in the sediment and continued to exist even after repeated centrifugation. Those coated at low number of layers, however, did not yield observable pellet after centrifugation.

† CaCO_3 is among the commonly used templates for capsule formation, apart from MF.

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Figure 5.5 – SEI of bare (a), coated particles (b), microcapsules (c,d), and optical micrographs of microcapsules (e,f). The number of layers except bare particles is 100. The ‘buried’ appearance of the capsules was caused by PLGA. PLGA was unable to be completely removed from the capsule surface with > 10 repeated washings in ACN. In both types of imaging system the collapsed capsule folds and creases are visible.

Figure 5.6 – Patches of films from dissolution of PLGA particles coated at lower layer number.

the shear force between them. The pH was also maintained at 7 during build-up*, and the subsequent immersion in pH 7.4 PBS does not create extreme changes in pH such that the PEM ‘de-laminates’, such as what occurs after immersion at pH 10, for example.¹⁰⁶

The release from the bare particles experienced a burst discernible for 2 days in the beginning, although relatively small (see Figure 5.7). This corresponds to ~15% of the total drug content. For both the control and the coated particle, this amount is lower, however, at ~5% of the release, corresponding to a 30% release reduction. From a measured loading of 3.4%, the difference in amount is actually 6.7% of the total drug, *i.e.*, release from uncoated particles – release from coated particles = 6.7% of total drug content.

At this point, it is important to note that the reduction in release after coating could be caused by drug loss, instead of by release retardation. This is what most likely happened to the control sample. We have charted the release profile as percentage of drug loading, and customarily it is intuitive to conclude that the release profile of the coated particles, which shows a reduced value, is the result of release retarda-

*pH 7 was chosen because at lower or higher pH, PLGA could potentially hydrolyze during the coating process, affecting the release.

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Figure 5.7 – Ibuprofen release from PLGA microparticles coated with PAH/PSS at 30, 70, 100 layers. The similarly lower amount of release after coating compared to control indicates that reduction comes from drug loss during coating. The control is a set of particles incubated in 0.5 M NaCl at pH 7 for the same duration as that of the coating of 100 layers. Where invisible, error bars are smaller than the symbols.

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tion, since it is charted in percentage (%). This however, is not necessarily true. As the release profile from the control sample demonstrates, the release is reduced even without any retardation from PEM, but merely from simple immersion.

Figure 5.8 – Hypothetical release curves before (A) and after (B) immersion. The reduction in this case is merely a result of drug being removed from the particle surface. The original total drug amount is 100 µg. After immersion, drug amount is 85 µg. The reduction due to drug loss is still registered as release reduction even when the release curve is presented in percentage, and this is merely caused by a wash-away effect of the drug on the surface

Consider also the following situation, illustrated in Fig 5.8. In a 100 µg-drug-containing particle, the release is 42.5 µg after 5 days, registered as 42.5%. Suppose that due to drug loss, the release is now reduced to 27.5 µg due to drug loss of 15 µg. This drug loss merely affects the drug located on the surface, and therefore reduces merely the burst release. The drug content now is 85 µg, and the release is now registered as 32.3%. At the outset, 32.3% compared to 42.5% drug released within the same period of time could appear as a reduction of release caused by release retardation, simply because this had been calculated based on the measured drug content. The reduction of release, even when charted in %, therefore, does not necessarily sig-

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nify that release is being retarded by the PEM, since, as we have shown, a simple immersion that reduces the drug loading also results in a reduction in %.*

In order to confirm that the reduction is caused by drug loss, then, the loading after coating must be lower than the original loading. The drug loading values of the coated and uncoated particles, however, show no significant difference. The release reduction is also merely 6.7% of the total loading, lower than the error in loading measurement, 10% (*i.e.* the standard deviation is about 10% of the loading value, and the release reduction is within this standard deviation). We therefore, could not conclude that the reduction of release is caused by reduced drug loading.

Quantitation of ibuprofen in the polyelectrolyte solution during coating to directly measure the loss amount is also not feasible due to interfering absorbance peaks of PSS near those for ibuprofen, at $\sim 220\text{nm}$ and $\sim 255\text{nm}$, which appear at greater amount due to its higher value of 1 mg mL^{-1} . Supported by the fact that the control exhibits a similarly reduced release, with a similarly insignificant difference in loading, the following schema are probable.

Schema 1 The PEM causes reduction in release by acting as additional diffusional barrier. The corollary by Figure 5.3 is that PEM on lower layer number is not as effective. Another corollary is that PEM permeability does not reduce any further once it reaches a certain number of layers, in this case at least 30, since the release curves from 30, 70, and 100 layers are virtually identical.

Schema 2 The PEM is not effective as additional diffusional barrier. In this case

*It matters not whether the drug release after loss is exactly the value after subtraction from the original. The argument still stands even if the new release is $> 15\mu\text{g}$, *i.e.*, release should still appear lower percentage-wise.

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the release reduction is due to the drug loss during coating, similar to the control sample. The corollary is that even a layer number of 100 is unable to effect any release reduction. The PEM is not sufficiently impermeable to ibuprofen.

Schema 3 Combination of the above 2 schema, with the release reduction caused by both drug loss and diffusional barrier of PEM.

In order to confirm the mechanism for the release reduction that covers the above possibilities, the following studies were conducted. Higher MW drug was used to increase diffusional barrier of PEM. The drug was chosen to enable drug loss studies. For these reasons FITC-dextran was chosen due to availability in many MW and potential evaluation of distribution inside the particles. As a comparison, release studies were also done on BSA, for which the drug loss during coating does not affect the release profile when PEM is permeable.

It might seem surprising that there is no significant difference from 30, 70, to 100 layers. This can be explained if the permeability differential of the PEM is taken into account. It has been reported that the permeability of PEM is decreasingly reduced with each layer addition, *i.e.*, additional layer is not as effective at decreasing the permeability further.¹¹⁰ In such case, the decrease in permeability in PEM build-up could converge rapidly to a certain value, beyond which no additional number of layer is capable of reducing the permeability. In this case, however, there is no reduction in the release because the converged permeability value is still insignificant compared to the permeability of the PLGA matrix itself. Should the PEM has a significantly reduced permeability, the convergence shall manifest itself in a limiting reduced release, that is the maximum reduction in release achievable regardless of the layer number. The

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alternative explanation, on the other hand, is much simpler. The PEM is merely not impermeable enough to molecules as small as 206 Da.

5.4 High molecular weight drugs

5.4.1 Morphology and drug distribution

To investigate the effect of MW on the release, FITC-dextran of different MW was encapsulated: 4 kDa, 70 kDa, 250 kDa. Drug loss studies were conducted to investigate the correlation between drug loss due to coating on the release.

The morphology of the particles and the drug distribution are presented in Figures 5.9 and 5.10 respectively. The surface of the particles becomes less porous as the MW of the drug is increased. At 4 kDa, the pores of the surface are clearly visible, whereas at 70 kDa, less pore is observed. At 250 kDa, finally, the surface is the smoothest with virtually no pores. Bright-field CLSM shows that the pores in particles containing 4 kDa FITC-dextran is smaller and distributed throughout the particle, whereas in particles containing 70 and 250 kDa FITC-dextran, the pores coalesce together, forming a few relatively larger cavities. This could be caused by better primary emulsion homogeneity with lower MW due to lower viscosity. The coalescing pores also explain the reason for less pores on the surface in particles of high MW drug. The pores are located inside.

From the CLSM images in Figure 5.10, FITC-dextran of 4 kDa in large particles is noticeably located at the surface rather than encapsulated within, unlike 70 kDa and 250 kDa FITC-dextran, which are distributed evenly throughout the particles except

Figure 5.9 – Morphology of small (a) and large particles encapsulating FITC-dextran of 4 kDa (b), 70 kDa (c), 250 kDa (d). The small particles shown here encapsulate 70 kDa FITC-dextran. The particles encapsulating the rest are similar in morphology.

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whenever there are cavities. This observation is also made on the smaller particles. For both 70 kDa and 250 kDa particles, the drug is evenly distributed, unlike the lowest MW FITC-dextran. One could observe from the small particles containing the low MW drug that due to the intense fluorescence coming from the cavities, FITC-dextran could be as well located on the surface of the cavity, which dissolves as soon as it is in contact with aqueous solution. The high background intensity is caused by the relatively low fluorescence intensity from 4 kDa FITC-dextran. Compared to the higher MW FITC-dextran, the former exhibits less fluorescence intensity. Note that for this imaging, the particles were dispersed in PBS.

5.4.2 Drug loss amount during coating

In order to test for the drug lost during the coating process, each polyelectrolyte was quantitated for FITC-dextran amount. A control sample was also provided for a measure against simply ‘washed’ particles, done in PBS. The quantitation was done by calibrating FITC-dextran in each polyelectrolyte, control, and rinsing solutions immediately after coating and measurement of fluorescence activity afterwards. The result for all sets of particles is presented in Figure 5.11. The low layer number was chosen in order to distinguish the effects of retardation by PEM and drug loss.

For a preliminary study, the effect of freeze-drying on the coating process was monitored. For this, two sets of particles can be distinguished. One coated before being freeze-dried, the other, afterwards. It is clear from Figure 5.12 that the amount of drug loss is greater when the particles are coated after freeze-drying, in conjunction with pore formation during freeze-drying.¹⁶⁷ Subsequently in this chapter, the

Figure 5.10 – Distribution of FITC-dextran of 4 kDa, 70 kDa, and 250 kDa encapsulated in small (A), and large (B) particles with their bright-field images (C). Low MW (4 kDa) FITC-dextran is distributed more near the particle surface in both small and large particles than in the core, unlike 70 kDa and 250 kDa FITC-dextran.

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particles are coated after freeze-drying unless otherwise indicated.

Regardless of the size, the amount lost is highest at the first coating step, with the next coating steps producing negligible amount of loss. Drug on the surface readily dissolves upon contact with the polyelectrolyte solution, noting the hydrophilicity of FITC-dextran. Smaller particles, however, release less drug during the coating process (see Figure 5.13). This is likely due to the presence of large pores in large particles. The loading difference does not justify such reduction in drug loss.

On both small and large particles, amount of drug loss is greatest with the smallest FITC-dextran, 4 kDa, at 12% of the total drug content for large particles, while the rest of the FITC-dextran exhibits loss at < 6%. Although comparison on large particles between these values and the loading data suggest that this is caused by a higher loading, smaller particles also lose the most drug at 4 kDa, even when the loading between FITC-dextran MW is comparable. This indicates that the high amount of loss is not due to the high loading. The fact that the drug is distributed near the surface aids in explaining the high amount of loss, whereas higher MW FITC-dextran is distributed evenly throughout the particle. We had further encapsulated 4 kDa FITC-dextran at lower loading values and the drug loss (in percentage) during coating is not significantly different from those at lower loading values, confirming the above explanation.

5.4.3 Correlation between drug loss and release

The release from all particles exhibits the same features, with burst in the beginning without any sustained release afterwards for at least 3 months with 250 kDa. FITC-

Figure 5.11 – FITC-dextran of 4, 70, and 250 kDa lost during coating on small (**A**) and large particles (**B**). Loss is greatest with the smallest FITC-dextran. Note that the percentage is calculated based off the total drug content.

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Figure 5.12 – Effect of freeze-drying on the amount of drug lost during coating. Large particles employing FITC-dextran of 4 kDa was used in this instance.

Figure 5.13 – Comparison between particle loading (A) and total amount of FITC-dextran lost during coating (B) for both small and large particles. Drug loss is not caused by the loading, since 4 kDa FITC-dextran has the highest loss on both particles. The drug loss is calculated as a percentage of total drug loading.

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dextran has been reported to exhibit the same release pattern elsewhere.^{168,169} The release was continued for only large particles containing 4 kDa FITC-dextran because of its small size and therefore faster rate. However the release remains tapered until 100 days (see Figure 5.14). We have previously explained that the reduced release could follow at least 2 basic schema with one additional combination of the two.

Figure 5.14 – Release of 4 kDa FITC-dextran from large particles in PBS (A) and in PBS with 0.01% (v/v) Tween® 20 (B). Particles of smaller size or encapsulating FITC-dextran of other MW also show a similar burst in the beginning without any diffusion phase.

Because of the shape of the release, there is no difference in the release amount from day 1 onward until the next degradation phase. Until then, the release comes from burst release since day 1. The release reduction here, in the case of FITC-dextran then, can be defined simply as the reduction in burst release in release profiles between coated and uncoated particles. Depending on the sample, this burst can be in the order of one or a few days, and works only with release profiles that are distinc-

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tively composed of burst and degradation phase, between which barely any release is observed. Mathematically, it is

$$\text{Release reduction (\%)} \equiv \text{Burst without coating (\%)} - \text{Burst with coating (\%)} \quad (5.1)$$

For large particles containing 4 kDa FITC-dextran, the amount of release at day 1 for uncoated particle is 16%; for 70 kDa, 6%; and for 250 kDa, 4%. The release reduction, *i.e.*, the difference between these values and those from coated particles at 2 layers, is $11.2 \pm 1.3\%$, $4.0 \pm 1.0\%$, and $3.0 \pm 0.6\%$, respectively. That the values are not significantly different from the drug loss during coating, *i.e.*, $12.0 \pm 1.0\%$, $5.6 \pm 1.0\%$, and $3.2 \pm 0.4\%$, respectively, indicates that reduction in release is simply caused by drug loss (see Figure 5.15).

Figure 5.15 – Comparison between release reduction and drug loss. Drug loss and release reduction are not significantly different for each FITC-dextran MW

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It is known that freeze-drying causes burst release in FITC-dextran-encapsulating particles, and that the amount of drug loss is in proportion with the creation of accessible pores during freeze-drying.¹⁶⁷ This results in the drug loss during coating, which was done after freeze-drying. In order to circumvent this and reduce the amount of drug lost during coating, coating was done immediately after the particles were harvested. Release medium was also added with Tween[®] 20 in order to increase the rate of release.

For large particles containing 4 kDa FITC-dextran, the drug loss decreases from 12% to 8.7% within the same number of layers, showing that the drug loss could be minimized when freeze-drying is not employed. Upon increment of layer number to 8, the loss amount reaches 27%. At such high amount of loss, unlike particles with lower loss, the measured loading values show significant difference after coating, with the loading values being $1.8 \pm 0.3\%$, $1.4 \pm 0.2\%$, and $1.1 \pm 0.1\%$ for uncoated, 5 layer, and 8 layer-coated particles, respectively. Following drug loss amount, the release is similarly reduced by 22%, based on the original loading value (see Figure 5.16). In order to avoid confusion, release curve is plotted in μg , and the percentage is based on the original drug loading.

In contrast with high drug loss from 4 kDa FITC-dextran containing particles, for large particles containing 250 kDa FITC-dextran, the amount of loss is 0.6% at 8 layers (see Figure 5.16). The loading values, as a consequence, show no significant difference after coating, and the release reduction is, in proportion to the 0.6% drug loss, at 0.2%. Both particles coated without freeze-drying therefore, exhibit release reduction in proportion to the drug loss, similar to particles coated with freeze-drying. In

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all samples regardless of the coating method, the amount of drug release is either approximated by the amount of loss, or insignificantly different, leading to stronger indication that release reduction is caused by drug loss even at higher layer number.

As a control, 4 kDa FITC-dextran containing particles were washed with PBS. The release curve is plotted in Figure 5.17. The burst phase results in 32% release, and the control sample at 17% of the original drug content. Since no other mechanism but drug loss is working on the control sample, reduction in release must only occur through drug loss during washing. The drug lost was quantitated at 15%, and the reduction in release approximates it, at similarly 15%.

Comparison between the drug loss amount and release reduction on samples coated with or without prior freeze-drying, including the control sample, therefore, corroborates Schema 1, which explains the drug reduction on both ibuprofen and FITC-dextran particles through drug loss. In these systems, therefore, PEM is not impermeable enough to retard the drug. The drug loss originates from drug on the surface. Upon contact with washing/coating solution, the drug immediately dissolves from the surface, assuming that burst comes only from the drug on the surface. The burst afterwards is lower because the amount of available drugs for burst has decreased. This is easily seen in FITC-dextran, where the release shape is clearly distinguishable between phase I and III without any sustained diffusional release in-between. The release from degradation phase, therefore, should not be affected because it comes from the core, as discussed in the Literature Review.

Figure 5.16 – Release reduction of 250 kDa (A) and 4 kDa FITC-dextran (B) from large particles incubated in PBS with 0.01% Tween® 20. Coating was done immediately after harvest, with freeze-drying following. For 4 kDa FITC-dextran, the reduction in release is 22% at 8 layers based on the original drug content. The drug loss is 27%. In comparison, for 250 kDa FITC-dextran, the reduction is 0.2% and the drug loss is 0.6%.

Figure 5.17 – Release of 4 kDa FITC-dextran from the control sample incubated in PBS with 0.01% Tween® 20. The reduction is 15%, corresponding to 15% drug loss.

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5.4.4 Comparison with films

The same mechanism for release reduction is also found in FITC-dextran-encapsulating PLGA films. For 4 kDa FITC-dextran, there is no significant difference between the uncoated and coated films. Compared to the particles, where the reduction is more due to lower MW, there is no reduction due to coating, most likely since particle fabrication and freeze-drying produce open pores, creating greater access to encapsulated drug.¹⁶⁷ The result is presented in Figure 5.18.

For 70 and 250 kDa FITC-dextran, the same reduction in release from films as from their particle counterpart was found (see Figure 5.18). The release curve is shifted downwards, and with a control sample only washed in PBS using the same routine as the coating procedure, was found to be reduced to the same amount of those coated, showing that, similar to particles, release reduction in films is caused by drug loss during coating.

5.4.5 Optimized release characteristics from microparticles

Due to the drug loss which reduces the release of FITC-dextran, BSA was employed to effect a release curve unaffected by such loss and which exhibits a release curve without a strictly separated burst and degradation phases. With a less distinct triphasic release, release retardation could be observed simply from the reduced slope of second part the release curve. Such reduced slope could not be observed in FITC-dextran because the release tapers off as soon as it undergoes burst release.

The objective of using BSA was therefore to enable monitoring of the release retardation without any interference from the effect of drug loss due to coating such

Figure 5.18 – Release of 4 kDa, 70 kDa, and 250 kDa FITC-dextran from PLGA films. Release is reduced by drug loss from the PLGA films during coating.

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as found in FITC-dextran, and to monitor the release retardation when the release is sustained. To achieve this, burst release must be lowered and drug release more sustained. Particles must also be higher in density with better encapsulation into the particle core.

BSA has been commonly used to model protein release from PLGA.^{17,119,149,170–173} It has long been reported that BSA remains the same in molecular weight after encapsulation (although its conformation is compromised), and only hydrolyses with the developing acidic micro-environment in the degrading particles.^{174–176}

In order to create higher density particles with smooth surface morphology and minimized burst, 5% NaCl was added into the external PVA solution for all the particle sets. It is well known that addition of NaCl reduces burst and porosity.^{47,177,178} As a control, less dense and more porous particles encapsulating BSA were also fabricated. The loading values are tabulated in Table 5.2.

Table 5.2 – BSA encapsulation in microparticles

Size (μm)	Surface morphology	Loading (%)
620	heterogeneous	2.2 ± 0.3
160	homogeneous	2.2 ± 0.2
60	homogeneous	5.0 ± 0.1
2	homogeneous	2.6 ± 0.1

The morphology of each particle set is laid in Figures 5.19 and 5.20. The particles without NaCl addition are not spherical, with flat facets on the surface as a result of centrifugation, an indication of a less dense structure. The pores are of the order of < 5 μm. Those with NaCl addition, on the contrary, exhibit smooth surface characteristics with few to no pores on the surface, which have been reported in some BSA-encapsulating particles.¹⁷⁷ The morphology after coating remains the same for

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all particles. Previously coatings at larger layer number up to 100 have been shown to exhibit the same morphology as uncoated particles in ibuprofen-encapsulating particles.

Figure 5.19 – Morphology of porous particles of 620 μm encapsulating BSA (a,b) and its release profile (c). The morphologically heterogenous surface (a) of the particles is the result of pores (b). The inset shows initial time points. Although 4 layers show significant difference at early time points, the values are not significantly different from the control sample.

Due to its size, particles of 2 μm size agglomerate during coating. The particle size was monitored with DLS and was found to exhibit increase in aggregate size up to

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Figure 5.20 – Morphology of particles of 2 μm (C), 60 μm (B), 160 μm (A), and their release profiles. Square symbols indicate bare particles, whereas circles indicate particles coated at 4 layers. Release retardation is observed only at 2 μm particle size. Modified from a Creative-Commons-Attribution licensed article.¹⁷⁹

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6 μm at 3 layers, but returns to a slightly larger size of 3 μm after 4 layers of coating. The matter of agglomeration is dealt with more heavily in the subsequent chapter.

Release from porous particles exhibit a similar characteristics with those of FITC-dextran. Although release from particles of 4 layers is lower at initial time points compared to uncoated particle, it is not significantly different from the control sample (see Figure 5.19). This indicates that the lower release from coated particles result from drug loss during coating.

Similarly, the release from smooth particles, shown in Figure 5.20, shows that coating does not effect any difference on the release for particles of size 60 and 160 μm , with non-existent BSA lost during coating. This shows that the absence of proper release retardation by PEM is not strictly due to incomplete coverage of PEM owing to inaccessible pores during coating, since particles of no pores also experience no reduction in release. As a note, burst is also non-existent on both particles even when the drug loading is compared to FITC-dextran encapsulation, and not surprisingly, the release is slower and more sustained compared to the porous particles. This is more prominent on particles of 160 μm , which shows an extended induction time of at least 10 days, due to its larger size and lower loading compared to 60 μm .

In a simple diffusional release, smaller particle size typically results in faster release due to shorter diffusional pathway. Because of the lower loading however, the release amount is also lower.¹⁸⁰ Release was also done in pH 9.6 buffer for other particles, but there is no significant difference between bare, coated particles, and control sample for particles of 60 and 160 μm sizes.

We also note that for microparticles $\geq 60 \mu\text{m}$ during the release studies, no particle

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fusion was found in coated particles. Fusion occurred in otherwise uncoated particles. It is a well-known phenomenon,^{171,181,182} and is caused by the lowering of T_g due to hydration and degradation, which prompt chain re-ordering across the surface of the polymer due to increased chain mobility. That the coated particles do not experience this, then, is caused by the PEM barrier that prevents the polymer chain from being in contact with other particles. This phenomenon persists throughout all of the experiments, and indicates the persistence of PEM layer throughout.

Release reduction is only found at lower size of 2 μm (see Figure 5.20). The control sample shows higher release than the coated particle, and upon replacement of medium buffer into a higher pH at 9.6, BSA release increases approaching the level of uncoated particles, indicating that at higher pH BSA becomes more permeable. This is the first indication that release retardation works at smaller particle size.

It is also noted that during coating, particles underwent distinct aggregation at each layer, culminating at layer 3 at $\sim 6\text{ nm}$ (see Figure 5.21). At 4 layers, however, the particle returned to the level close to the original size. The change in particle size, from 2–3 nm could potentially lead to the reduction in release. This will be discussed more in the subsequent chapter.

Comparison can be made against reported works on PEM capsules that encapsulate BSA. These sometimes employ the commonly studied PAH/PSS,¹⁸³ or PAH/PMA,¹⁸⁴ but also natural polyelectrolytes such as PLL/chondroitin sulfate,¹⁸⁵ chitosan/dextran sulfate,^{139,186} or a mix of synthetic/natural polyelectrolytes such as carboxymethyl-cellulose/PAH,¹⁸⁷ with layer number typically at most 10 (5 pairs). A common feature of these capsules is that they release the load within hours (*i.e.* 5 h,^{139,184,186} 7 h¹⁸⁷),

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initiated with a burst followed by a very slow sustained release afterwards. The same is observed in PLL/chondroitin sulfate capsule, with a burst at 30–60 min, followed by tapered release.¹⁸⁵

In a work using PAH/PSS capsules, the closest to our system, for example, the burst is at 2 h at 35% release, followed by an almost flat release curve subsequently.¹⁸³ This is also similar to another PAH/PSS giant capsules (in the order of mm), with a burst of near 80% in 3 h,¹⁸⁸ followed by what seems like an incomplete release.

This release behavior is different from capsules containing small molecular weight drugs. In those capsules, release follows a single phase with a linear increase at the beginning without any burst nor incomplete release. The main cause seems to be, as far as diffusion is concerned, the fact that in those works working on small drugs, capsules are ‘loaded’ by coating the PEM on the drug crystal as a template, as opposed to these BSA-containing capsules, which undergo cyclical pH change to open, entrap BSA from the solution, and close at higher pH*. It is probable that more drugs are located in the walls of the capsules than within, causing the burst; whereas the sustained release comes from the BSA within. Incomplete release could then be caused by immobile BSA in the wall.

Regardless, the short time of release from these capsules suggests that the permeability of PAH/PSS capsules is not as small as to warrant an additional barrier for BSA release from the PLGA particles. Since permeability is, in capsules, directly proportional to the partitioning coefficient and diffusion coefficient, it could be caused then by either a relatively high diffusion coefficient in the PEM or a relatively high

*Note that these capsules also open and close with pH variation, with lower pH being an open state as opposed to higher pH

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partitioning coefficient. Due to the low thickness of the PEM, partitioning coefficient might be the dominant factor.

In the subsequent chapter, a probable explanation is offered for the virtually absent release retardation from larger microparticles encapsulating BSA as opposed to those at 2 μm .

Figure 5.21 – z-average evolution during coating of 2 μm particles encapsulating BSA

5.5 Conclusion

The series of experiments beginning with low MW drugs on fluorescein, ibuprofen, and FDA show that release is not retarded by PEM on micron-sized particles. Even with an increased PEM layer number to 100, retardation is not observed. In order to investigate the drug loss, FITC-dextran, a higher MW drug model, was employed.

The FITC-dextran thus employed however, shows that reduction in release, virtually observed from the outset, is concluded to be of drug loss origin during coating,

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regardless of the dosage form: particles or films. The drug loss studies show that the amount of loss is greatest at the first coating step, sensitive to freeze-drying, independent of loading, and not significantly different from the drug loss on large particles.

The implication of the above observation is that the currently reported *in-vitro* drug release curves in the literature are inadequate to report release retardation from release barrier when the drug loss during additional procedures to establish such barrier is not reported, or a control is not provided.

Only release retardation was observed in BSA, and even this at lower particle size ~ 2 nm, whereas particles above this size do not display any release retardation. This is the first indication that release retardation is dependent on the dimension of the carrier, even when the same PEM is used for coating.

Comparison of BSA release from capsules in the literature also shows that PEM might not be able to retard release from PLGA, since release from microcapsules is in the order of hours,^{184,185,188} whereas sustained release from PLGA particles is in the order of weeks to months. Indeed, only at low particle size is it able to have retardation, indicating a size-dependence of the release retardation, showing that regardless of the dominant factor of retardation.

CHAPTER 6

Polyelectrolyte Coating on Nanoparticles

6.1 Introduction

Our work on BSA using particles of different size shows that particles do not experience release retardation at higher particle size $\geq 60\text{ }\mu\text{m}$. Indeed, at micron-size level, to our knowledge, there is no report on release retardation by PEM coating on PLGA.

Only microcapsules are reported to have such full retardation, thus they are judged ‘impermeable’. This is shown by works on FITC-dextran post-loading via pH change,¹¹⁵ for instance. FITC-dextran of both MW 75 kDa and 4 MDa were able to remain in the capsules, or diffuse out depending on the pH-range. This observation, unfortunately, was done in time scale of hours and at zero ionic strength. It is known, however, that at higher ionic strength, the permeability of PEM is higher,¹⁸⁹ rendering any comparison of PEM permeability in those works and in ours difficult.

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Other works showing impermeability of capsules to PAH-FITC was also done within the time scale of 20 min.¹⁹⁰ These render attribution of ‘impermeable’ not applicable to our duration of sustained release. In fact, using a similar criterion to judge whether a capsule is ‘impermeable’, our drugs encapsulated in PLGA with such time scale would equally qualify as such, simply because through the same time scale, drugs remain within particle through CLSM visual confirmation, and this is without any PEM! At longer duration, however, it becomes obvious that such is not the case.

The problem, therefore, is that the word ‘impermeable’ has been liberally used simply to distinguish the vastly different release rate of the drug between two conditions (low or high pH, for example), and does not quantitatively describe at what rate the drug diffuses under both conditions. This ‘relative’ aspect of impermeability is further confirmed by the fact that most of these capsules judged impermeable were judged such through visual observation.

Since works on microcapsules have not shown release retardation quantitatively, whereas works on nanoparticles in core-shell form (not capsules) have shown some release retardation, for instance, on liposomes, as shown in our Literature Review chapter, we proceed to investigate release retardation on nanoparticles in this chapter.

We have shown in the previous chapter that at particle size of 2 nm, drug release starts showing retardation. Below this size, as discussed in Chapter 2, the literature is also abundant with evidence of such retardation, although the template and drugs used are different, as discussed earlier, even if PLGA was used.

In order to explain this, we attempted theoretical analysis assuming only the diffusional process without any other release-controlling mechanism. The objective is to

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explain the difference in release retardation due to size. Since surface area is directly related to size, we hypothesize that it is the surface area of the particles or films that distinguishes release retardation behavior between microparticles (as well as films) and nanoparticles. The former has much lower surface area compared to the latter.

The model drug for the nanoparticles in this chapter is BSA, due to its large size, hydrophilicity, and for further comparison with microspheres encapsulating the same. BSA has been incorporated in PLGA nanoparticles.^{191–193}

Moreover, for nanoparticles, thus far in the literature factors such as particle aggregation and drug loss during coating have not been discussed with respect to release retardation. As discussed in Chapter 5, drug loss affects the release even when the cumulative release is presented in percentage of total drug amount. With an increased surface area, the amount of drug loss during coating is expected to be even higher.

Most of the content of this chapter has been published in PloS ONE.¹⁷⁹

6.2 Experimental procedures

The particle fabrication follows the following w1/O/w2 procedure. For nanoparticles, w1: 0.4 mL of 30 mg mL⁻¹ BSA in PBS, O: 100 mg of PLGA 50/50 (IV 1.18 dL g⁻¹) in 4 mL of DCM, and w2: 10 mL of 1% (w/v) PVA 30000. Both emulsions employed 40% sonication for 2 min each, and evaporation was done by stirring at 1000 rpm overnight. To prevent loss of particles during handling and freeze-drying, as-harvested samples were directly coated and set for release without lyophilization beforehand. Due to aggregation in PBS, water was used for the control instead. The control samples underwent the exact treatment of repeated centrifugation and dispersion, only in

water.

For particles coated employing sonication, the following procedure was followed: For nanoparticles, w1: 0.2 mL of 120 mg mL⁻¹ BSA in PBS, O: 200 mg of PLGA 50/50 (IV 1.01 dL g⁻¹) in 4 mL of DCM, and w2: 10 mL of 1% (w/v) PVA 30000 and 5% (w/v) NaCl, with sonication of the first and second emulsion at 40% power for 2 min each, and stirring at 1000 rpm overnight.

Release studies for BSA-containing nanoparticles were done in 1 mL solution per sample in PBS, with replenishment rate of 0.5 per time point. Aliquots for measurement were re-centrifuged to remove any traces of particle at 13000 rpm for 15 min before being measured for BSA amount with BCA.

6.3 Size dependence of release retardation

It is customary to discuss diffusion-related phenomena in terms of flux, that is, the rate of mass transfer per unit area. This is analogous to drug release, in this context. And since flux (J) is disproportionate to layer thickness (l) at steady state according to

$$J = DK\Delta C/l \quad (6.1)$$

, where D is the diffusion coefficient, C is the drug concentration, and l is the layer thickness, we hypothesize that the increase in relative thickness compared to particle size (*i.e.*, l/R) will reduce the release even further.

When the particle size is reduced to sub-micron level, *e.g.* to 250 nm from 100 μ m, the specific surface area will have increased from only 6×10^4 m⁻¹ to 2400×10^4 m⁻¹.

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The relative thickness of the layer will also have increased by the same order of magnitude.

For diffusional release on composite geometry, analytical solution for the flux on steady state has been worked out.¹⁹⁴ What has *not* been calculated, however, is the ratio of the flux with coating against that without, which we are undertaking here. Although the problems were set in the context of heat transfer, it is analogous to the problem in mass diffusion, since both share the same form of elliptical differential equation.

Figure 6.1 – Schematics of PEM-coated particle

For convenience, flux here is simply defined as the mass transfer rate, not mass transfer rate per unit area as per literary convention. The assumption is simply that the flux is at steady state, without any accumulation of drug in the PEM. Drug diffusing from the particles move in in the same amount as that leaving the PEM. For a composite sphere, this flux, according to Gebhart, is

$$F = \frac{C_i - C_0}{R_T} \quad (6.2)$$

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where C_0 is the drug concentration outside and C_i the concentration inside the particles. The resistance R_T is the sum of individual resistance R ; for each layer

$$R_a = \frac{1/r_1 - 1/r_2}{4\pi D_1}, R_b = \frac{1/r_2 - 1/r_3}{4\pi D_2} \quad (6.3)$$

and so on for additional layers. The region from an arbitrarily chosen point r_1 in the uncoated particle to the surface of the particle is one resistance R_a , and the region from the surface of the particle to the surface of the PEM coating is another resistance R_b . In total there are 2 resistances. The total resistance is

$$R_T = \frac{1/r_1 - 1/r_2}{4\pi D_1} + \frac{1/r_2 - 1/r_3}{4\pi D_2} = \frac{D_2/r_1 - D_2/r_2 + D_1/r_2 - D_1/r_3}{4\pi D_1 D_2} \quad (6.4)$$

Substituting to Equation 6.2, the flux for coated particle then is

$$F_T = \frac{4\pi D_1 D_2 (C_i - C_0)}{D_2/r_1 - D_2/r_2 + D_1/r_2 - D_1/r_3} \quad (6.5)$$

Assuming initial time points where the difference of concentration between coated and uncoated particles at r_1 and r_3 is negligible, so that the difference in concentration in uncoated particle is also $C_i - C_0$, the flux for uncoated particle is simply

$$F_0 = \frac{4\pi D_1 (C_i - C_0)}{1/r_1 - 1/r_2} \quad (6.6)$$

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The ratio between the flux of coated (F_T) and uncoated particle (F_0) becomes

$$\frac{F_T}{F_0} = \frac{D_2(1/r_1 - 1/r_2)}{D_2/r_1 - D_2/r_2 + D_1/r_2 - D_1/r_3} = \left(1 + \frac{D_1}{D_2} \frac{1/r_2 - 1/r_3}{1/r_1 - 1/r_2}\right)^{-1} \quad (6.7)$$

$$\frac{F_T}{F_0} = \left(1 + \frac{D_1}{D_2} \frac{t}{r_3} \frac{1}{r_2/r_1 - 1}\right)^{-1} \quad (6.8)$$

with t being the thickness of PEM. This is the ratio of the flux at a certain time point between r_1 and the surface of the particle, and is descriptive only during the initial time points when $C_i - C_0$ is the same for both bare and coated particles. At later time points the ratio is underestimated by the equation, *i.e.*, the real ratio is higher than prescribed, since $C_i - C_0$ will become higher in coated particles than uncoated particles, due to slower release.

With $r_1 < r_2 < r_3$, this expression has a maximum of $F_T/F_0 = 1$. At $D_1 = 0$, it becomes a capsule and the ratio is 1, denoting no difference in release. At $t = 0$, coating does not exist and the ratio is also 1. When the particle size increases, *i.e.*, $r_3 \rightarrow \infty$, $F_T/F_0 \rightarrow 1$, and conversely, as $r_3 \rightarrow 0$, $F_T/F_0 \rightarrow \infty$.

If the dependence on size follows the above scheme, it is expected that the release retardation in nanoparticles is even greater compared to microparticles. The above elaboration was done without any regards to the partitioning coefficient in order to simplify the final analytical expression. With partitioning coefficient, a similar dependence should still be observed.

6.4 Particle aggregation

BSA-loaded nanoparticle formation was confirmed through size measurement with a z-average of 330 nm. The size ranges from 100 nm to less than 1 μm (as shown in Figure 6.2). The particles exhibit an encapsulation efficiency of 68%, and a ζ -potential of -25 mV . The ζ -potential of particles coated with PLL/DES showed considerably smaller value than those coated with PAH/PSS, because of the lower charge density. For comparison, the absolute value on PAH/PSS could reach as high as 40 mV, while it is only 15 mV on PLL/DES.

Figure 6.2 – Typical number particle size distribution of BSA-loaded PLGA nanoparticles

As-harvested PLGA nanoparticles were relatively stable in the PVA solution. After a few rounds of centrifugation and re-dispersion in water, however, particles start to

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aggregate, increasing to a stable size of ~ 400 nm, after which the particles no longer increase in aggregate size. Greater amount of aggregation starts to form with polyelectrolyte coating, with the size increasing to as large as $4\text{ }\mu\text{m}$, in the most extreme cases, with PAH/PSS.

Note that although aggregation exists in both microparticles and nanoparticles, the former, which is in the order of $60\text{ }\mu\text{m}$, consists of PLGA fusion above its T_g at $37\text{ }^\circ\text{C}$. It does not occur spontaneously, rather, only after a few days, and does not affect coated particles with number of layer as few as 4 in the studies, due to barrier of chain diffusion by virtue of the PEM. On nanoparticles, in contrast, the aggregation is due to the charge of the respective particle, the interaction between the polyelectrolyte chain on one particle and another, and occurs instantaneously after dispersion in the respective medium.

PAH/PSS samples without any sonication yields the greatest aggregation, reaching macroscopic size in water visible to the un-aided eye at the 3rd layer. This renders any measurement for aggregation-index not representative, due to the reported particle size originating only from those that are freely dispersed. When coating was done at pH 9 with the same polyelectrolyte, the size increased up to 3 layers at $4.8\text{ }\mu\text{m}$ and reduced to $2.6\text{ }\mu\text{m}$ at 4 layers. This was done in water, however. The aggregate size in PBS is even higher. In order to monitor the size progression during coating, therefore, PLL/DES was employed.

The progression of the aggregate size for PLL/DES coated particles is shown in Figure 6.3. When coated with PLL/DES, particles were dispersed well and produced either negligible macroscopic aggregation next to none. Macroscopic aggregation is a

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significant factor in reducing the release due to the much less surface area it creates on the resulting aggregates.

PLL and DES itself have been employed for PEM assembly with each other or other polyelectrolytes for cell-related applications, such as guiding cell differentiation,^{195,196} changing cell behaviour,¹⁹⁷ or creating enzymatically degradable microcapsules.¹⁹⁸ The suitability of PLL/DES in this study extends to the fact that just as PAH/PSS, they are a weak and strong polycation and polyanion combination, respectively. The polycation has a similar pK_a values to PAH (~ 9),^{199,200} while the polyanion is strongly ionized throughout the pH region of interest here ($pK_a < 2$),²⁰¹ similar to PSS.

As seen in Figure 6.3, particles coated with PLL/DES follow a similar trend, with the aggregate size relatively unchanging up to 2 layers, followed with a sudden jump at the 3rd layer. Afterwards the size remains the same or decreases to its previous value. A similar size progression has also been reported on liposomes and nanoparticles.^{202,203}

For comparison, with only 2 layers consisting of PAA/PAH, it was reported that liposome aggregate size increased from ~ 250 nm– ~ 520 nm.¹³³ On smaller liposomes, the size increased from ~ 110 nm to ~ 140 nm with PAH coating and to ~ 230 nm with a final PAA coating.¹³⁶

Regardless, the aggregation level found here is much lower than PAH/PSS without any sonication. No macroscopic aggregate was found, unlike PAH/PSS. This makes correlation with the release curves possible.

As a side note, aggregation due to polyelectrolyte coating derives from a different

Figure 6.3 – Release profiles of BSA from nanoparticles coated with PLL/DES built at pH 4 (top) and 9 (bottom), and their aggregate size evolution. The release profile of the uncoated particles is shown in Figure 6.7.

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origin from uncoated particles. The latter is governed by the classical DLVO theory, whereas the former is in the first place due to patches of uneven polyelectrolyte ‘decoration’ on the surface, creating regions with non-uniform sign of charge.^{129,204} This creates electrostatically attractive forces between patches of surfaces that are dissimilar in charge, and is the origin of aggregation in polyelectrolyte-coated particles. It is known, for example, that gold nanoparticles,²⁰⁵ latex particles²⁰⁶ and liposomes²⁰² aggregate in the presence of polyelectrolyte. This aggregation, therefore, is transient in so far as it depends on the ionic strength, particle concentration, pH, etc. Further dilution, for instance therefore, could change the nature of the aggregation.

Although this phenomenon has been investigated extensively in a situation where aggregation occurs during incremental polyelectrolyte addition into nanoparticle suspension, and is dictated by the ζ -potential—having maximum aggregation near neutral charge—an increased, irreversible aggregation could occur due to maximum proximity between particles effected by centrifugation. Even without polyelectrolyte treatment, PLGA nanoparticles became aggregated with repeated centrifugation, with an increase of size from 330 nm to 400 nm after 2 rounds of centrifugation.

It was reported that closer inspection on the morphology of PEM coated on polystyrene nanoparticles revealed larger thickness and rougher morphology as the pK_a value is approached during coating, resulting in high aggregation.²⁰³ This could explain why layer 3 for PLL/DES coating has the highest aggregate size. At layer 1 and 2, the PEM film is not thick enough to entangle particles together. After addition of the 4th layer, the roughness reduces and so is aggregation. That PAH has high aggregation rate has also been reported, though in comparison with PAA.²⁰³ The higher

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aggregation at the 4th layer at pH 9 compared to 4 is then due to the pH nearing pK_a of PAH.

For particles coated by employing sonication during re-dispersion, the size progression is continuous without any jump or reduction. It is noteworthy that sonication is able to reduce the aggregation level of PAH/PSS coating, which produces macroscopic nuggets, to that of PLL/DES without sonication, without any macroscopic nuggets and at a maximum of 2 μm aggregate size. When re-dispersion is not assisted with sonication, the size progression exhibits a jump and reduction in size, and reaches 4 μm , compared to 1.9 μm with sonication. At the 3rd layer, however, the SD is higher, as well as the PDI. It is possible that at this layer, particles aggregate similarly to higher level, but sonication breaks apart aggregates non-uniformly, resulting in higher PDI.

6.5 Drug loss during coating

As discussed in Chapter 5, BSA lost affects release even if cumulative release curve is calculated based on the loading. To this end BSA amount lost during coating was quantified using calibration standards in its own respective solutions. PAH especially presents another potential complication due to insoluble complex formation with BSA. This occurs, however, at comparable BSA/PAH ratio, and reduces significantly when either component is added or removed from the solution.²⁰⁷

Visible complex formation also forms when in mixture with BCA reagents, possibly due to the change in ionic strength, but disappears upon incubation at 37 °C. The calibration curve however, although significantly higher than in PBS at low con-

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centration ($< 37.5 \mu\text{g mL}^{-1}$), still retains its linearity, and converges with that in PBS at high concentration. Variations due to pH were also anticipated, with pH showing no significant difference on the calibration curve at the range found in the coating processes. PAH concentration during coating was assumed to remain constant due to excess, after taking into account an approximate Γ value of 0.3 mg m^{-2} .^{208*}

Due to its much increased surface area, BSA quantification becomes more significant during the coating process, and the reduction in release could be caused by a simple reduction of drug amount. For this reason coating process was monitored for drug loss amount. It was expected that the amount of BSA lost could be exorbitant. The amount however, is maximum across our different samples only at 8% loss for PLL/DES coating. For other coating conditions, the values are provided in Figure 6.5, with the lowest being PAH/PSS coating at pH 4 and 9, at 3.7%. The amount of BSA lost is also greatest at the first few steps, and decreases with increasing layer number (see Figures 6.4 and 6.6). Attempts to reduce loss by pre-coating it during fabrication with PAH in the outer PVA solution, for instance, did not succeed because of minimal BSA encapsulation as a result. In any case, the total drug lost during coating of all the samples average to a value of 5.4%.

Note that contrary to FITC-dextran, the amount lost during coating is significantly higher than the control (see Figure 6.4). Although FITC-dextran control was done in PBS and not water, thereby rendering disagreement in data not fully comparable, it is suspected that BSA, being charged, desorbs from the particle surface more readily by electrostatic interaction with polyelectrolyte solution, whereas the same does not

*Values were taken from measurements on silica at pH 5. The value at pH 9 is about twice as much. For other values, see Ruths *et al.*⁷⁹

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Figure 6.4 – Comparison of BSA lost during coating with PAH/PSS at unadjusted pH (solid) and with repeated washing with water as a control (stripe)

occur in water.

Figure 6.5 – Effect of polyelectrolyte type during coating across different pH values on BSA loss. N.A. indicates that pH was not adjusted.

Furthermore, depending on the coating pH, BSA loss during the first polyelectrolyte adsorption could be either higher or lower than during its washing step. At

Figure 6.6 – Comparison of BSA amount lost at pH 4 and 9 in PAH/PSS and PLL/DES. Note the decreasing amount of loss at pH 9 after the first layer, in contrast with that at pH 4.

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pH 9, BSA amount lost during the first PAH adsorption is 2.4%. The subsequent washing step has the value drop to 0.2%. At pH 4, in contrast, the amount increases from 1.4% to 1.9%. The same trend is also found during PLL/DES coating, where BSA lost decreases during subsequent washing step only at pH 9, but not 4. This could be attributed to the pI of BSA, ~ 4.7 ,²⁰⁹ below which it acquires a positive charge. At pH 9 when BSA is negative, it could be more easily bound to the previously adsorbed positive PAH or PLL, hence the lack of BSA detected during the subsequent wash. Conversely, at pH 4, the reverse occurs.

The amount lost in PLL/DES is also greater than in PAH/PSS at any pH. It appears at the outset that the higher the lost amount, the higher the reduction, presuming the reduction in release is caused by a reduction of BSA amount in the particle. This however, is not true, as shown by correlation of drug release and drug loss on particles coated with PAH/PSS and PLL/DES.

PAH/PSS coating, which produces the lower amount of lost during coating compared to PLL/DES, has higher release reduction (higher release retardation) (see Figure 6.7). If release reduction were caused simply by drug removal during coating, the amount of release retardation should be lower than PLL/DES.

Comparing the release from PLL/DES coated particles at pH 4 and 9 also show that although pH 4 coating results in higher drug loss (see Figure 6.6), the release reduction is actually lower than pH 9 (see Figure 6.3), which has lower drug loss.

Two factors may contribute to this effect: the difference in permeability between PAH/PSS PEM, and the difference in aggregation caused by the two different PEM. It is conclusive however, that the reduction is not caused by drug loss, since it is also

supported by the fact that the reduction in burst release at time zero is $\sim 35\%$ in absolute amount for PLL/DES, and almost completely for PAH/PSS, compared to the amount of drug loss at a mere 8%. Such loss, therefore, could not explain away the reduction on its own.

6.6 Drug release from coated nanoparticles

Figure 6.7 shows the release curves of both the uncoated and coated particles with PAH/PSS and PLL/DES at unadjusted pH. When uncoated, the initial release when immediately dispersed in PBS was already 40%, and increased sharply with only one hour of incubation at 37 °C. This indicates that most BSA is located on the surface, or very near to the surface, but still bound strongly enough that repeated washing with the control sample could not remove significant amount of BSA.

From the same figure, it is apparent that the release reduction with PLL/DES coating is lower than PAH/PSS coating. Initial time points show that PLL/DES releases $\sim 40\%$ compared to its bare particles. Both are coated without adjusted pH at 4 layers. In contrast, PAH/PSS hardly releases any. Such a marked difference cannot simply be attributed to the amount of BSA lost during the coating process, because PLL/DES showed greater loss during coating (Figure 6.5).

For more details on each layer, the accompanying reduction of release up to 4 layers is presented in Figure 6.8 for PAH/PSS with unadjusted pH. The release is reduced through addition of PAH layer, while not as much through the addition of PSS layer, as seen by the relative reduction by the 2nd and 4th layer from the 1st and the 3rd, respectively. This pattern is repeated again with PLL/DES coating.

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Figure 6.7 – Release curve comparison of BSA between uncoated (squares) nanoparticles and nanoparticles coated with PLL/DES (triangles) and PAH/PSS (circles) at 4 layers with unadjusted pH. PAH/PSS suppresses release more than PLL/DES.

Figure 6.8 – Effect of layer number on release curve of BSA with PAH/PSS at unadjusted pH. For comparison, the release profile of uncoated particles is shown in Figure 6.7.

(a)

(b)

Figure 6.9 – Comparison of BSA release between uncoated and coated particle with sonication during the coating process, and its aggregate size evolution. Particles continue to aggregate up to 4 layers but without any significant difference in release.

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Due to the presence of macroscopic aggregation during the coating process with PAH/PSS, and to determine the effect on the release, particles were coated by re-dispersion in sonication. To reduce drug lost during coating, the particles were made with an additional 0.5 M NaCl into the external PVA to create a more dense particle with less burst. These are coated with PAH/PSS, and with the whole procedure including fabrication method, the resulting particle size after coating could now be measured electrophoretically. The original particle size before any coating is $\sim 400\text{nm}$. After 4 layers of coating, the size reaches $\sim 2\mu\text{m}$ (See Figure 6.9). The drug release shows that the five-fold increase in particle size does not influence the drug release significantly. This indicates that aggregation has little impact on the release at a five-fold increase of the particle size. The low release retardation, then, originates from the sonication process which effectively reduces the amount of polyelectrolyte adsorbed onto the particles.

In order to investigate the effect of assembly pH on the release retardation, PLL/DES was used, since it creates less and quantifiable aggregation than PAH/PSS. The effect of pH on the release profile of the particles is shown in Figure 6.3. Note that in all instances, the release profile of the uncoated particles is much higher (see Figure 6.7). The release is noticeably lower in particles coated at pH 9 due to the increased thickness of the PEM made near the $\text{p}K_a$ of the polyelectrolyte. With less charge, more polyelectrolyte is needed in order to invert the charge of the previously built layer, resulting in higher thickness.

Although it is not necessarily true that higher number of layers produces a lower release curve, *e.g.*, from 1–2 layers, the increase is either lower when the layer is ter-

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minated by the same polyelectrolyte, *i.e.*, 1–3, 2–4, or not significantly different. This is so regardless of the aggregation level. For instance, at build-up pH 4 where the aggregation is greater at layer 3, compared to the release profile at build-up pH 9 where the aggregation is similar for both layer 3 and 4. Regardless, layers terminated by the same polyelectrolyte yields lower release with higher layer number, or without any significant difference. In other words, a change in only one layer might not result in a reduced release, but additional 2 layers make it so.

That additional layer might not significantly reduce the release is also found in PAH/PSS coating (see Figure 6.8), where the difference in release retardation is with addition of 2 layers, as well as with liposomes, where those coated with (ALG/CHI)₃ releases higher amount of BSA than with ALG alone.¹¹³ The mechanism for this thus far is unknown, although this in effect means that an increase in layer number does not necessarily increase the retardation.

If the release is limited by the partitioning of the film, it means that significant difference in the partitioning is only resulted with a difference in one bi-layer. The consistent result of reducing release at any build-up pH with one bi-layer demonstrates this.

The difference in behavior of release retardation of PAH/PSS and PLL/DES might be attributable to the hydration of the PEM. It is known that polysaccharide-based PEM is highly hydrated.²¹⁰ It has also been reported that polyelectrolytes, though soluble on its own, could act as hydrophobic substance once assembled, demonstrated by preferential partitioning of curcumin into the film.²¹¹ Due to the higher hydration of PLL/DES, PLL/DES could be more hydrophilic and the partitioning of BSA into

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the layer higher than PAH/PSS. BSA, therefore, more easily partitions into the PEM coating than PAH/PSS.

6.7 Conclusions

The size dependence has been explained by a steady-state analytical expression of the ratio of the mass transfer of the coated *versus* uncoated particle, which describes a size dependence due to relative thickness of the PEM coating. The study on nanoparticles was done in order to show that at this level, retardation works due to the higher relative thickness. This same dependence should still be observed even when partitioning coefficient is taken into account.

BSA amount lost during the coating process reaches as high as 8%, but can be minimized by creating higher density particles with less burst. Furthermore, we have shown that the release retardation is not caused by the drug loss amount during the coating process.

We managed to de-couple the effect of aggregation from the effect of PEM on release retardation by showing that aggregated particles do not show release retardation. The absence of release retardation accompanied by aggregation indicates that aggregation is not effective in reducing the release. This indicates that release from nanoparticles is hindered in the presence of PEM, which shows greater impermeability by PAH/PSS pair than PLL/DES.

The release profiles of the coated nanoparticles also show that with PLL/DES, a dependence on the pH can be observed, with higher retardation at higher thickness and lower retardation otherwise. This is attributed to the increased thickness of the PEM

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at higher pH nearing the pK_a of PLL. With an increase in 2 layers, the amount of retardation is more prominent than additional 1 layer, where it could be negligible. This is observed in both PAH/PSS and PLL/DES, although the effect is more prominent in PAH/PSS. We have explained the difference in release retardation of both PAH/PSS and PLL/DES by the difference in partitioning. PAH/PSS, due to lower hydration, could be more hydrophobic than PLL/DES.

We have, therefore, demonstrated that the same principle in PEM build-up on typical substrates such as silicon and glass could be applied on PEM build-up on PLGA nanoparticles. Although the work here describes only pH dependence, a variety of other factors that could change the thickness of PEM built on PLGA could also potentially affect the release, such as ionic strength or other polyelectrolyte pairs, notably exponentially growing ones.

CHAPTER 7

Summary and Recommendations

We have investigated the potential for PEM on PLGA particles using several drugs. Release retardation is not found in particles encapsulating low MW drug, either hydrophilic or hydrophobic. Even with an increased number of layers, the same release curve is yielded. With higher MW model drug such as FITC-dextran, retardation is also not found.

Release retardation on FITC-dextran containing particles come about through drug loss during coating, which is affected by the MW of the drug, and results in simply a lowered release profile. This same phenomenon is found in not only particles but also PLGA films containing FITC-dextran.

Only with BSA at lower particle size is release retardation found. This is even more evident at nanometer-sized particles. Due to the shape of the release profile of BSA, quantification of the release retardation is not as simple as the difference between burst release of coated and uncoated particles, since BSA particles do not show a burst phase distinct from diffusion and degradation phase.

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Comparison between BSA and low MW drugs with different MW enable distinction between partitioning coefficient and diffusion coefficient as the dominant factor for release retardation. Unfortunately at the nanoparticle level, we could not achieve good encapsulation with ibuprofen due to short and complete burst within a short time span. The distinction between the role of diffusivity and partitioning therefore, has not been clarified.

We have attempted to explain the difference in size in terms of pure diffusion. Our argument is that at lower particle size, the relative thickness between the particle and the PEM is greater than on microparticles. This results in the release retardation from coated particles. At higher particle size the relative thickness decreases to negligible value, rendering difference between them also negligible. This effect is not only found in particles, since films do not exhibit the same retardation either.

When there is a difference, however, it is due to drug loss during the coating process, as shown by studies on FITC-dextran. We have shown that the drug loss could lead to the wrong conclusion of similarly retarded release. With closer inspection, it becomes clear that drug loss during coating is easily achieved by simple immersion in washing solution or PBS. For lower MW drug this is not an issue, since the release from the particles themselves does not seem to get affected even if drug is lost during coating.

In the beginning we have stated that the objective of the study was to determine the range of release retardation of PEM-coated particles, to understand the factors affecting the release retardation, and to develop a reasonable explanation of such retardation behaviour, with partitioning coefficient as most likely the dominant factor

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for limiting the drug release.

In response to our objective, we have provided a sketch of the overall picture of release retardation on PLGA microparticles. Although not all factors have been completely described, for instance the distinction between the role of partitioning coefficient and diffusivity, which we could not obtain in our study with drug polarity on microparticles, we have, in summary, found the following.

1. Particle size strongly influences the retardation, and the range is from nanometer to submicron particles with BSA, regardless of drug polarity
2. For microparticles which are not retarded, reduction is caused by drug loss, which in turn depends on the MW of the drug
3. For nanoparticles, the release is affected by the build-up pH of the PEM and the polyelectrolyte type. The size dependence behaviour has been explained through a simple steady-state description with diffusion.

Based on these results, it is recommended that release studies employing any sort of coating report the drug loss during said process, and that PEM coating be used for particles of small size to achieve a more sustained release. For PEM application on particles that does not need adjustment in the release kinetics, particles of larger size will not have its release curve changed, for instance for surface modification or cell recognition application, since at this size the relative thickness of the PEM is too small to effect any retardation.

The capability of PEM to be built in response to pH on PLGA particle is also useful for other changeable build-up factors, for instance with other polyelectrolyte pairs

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which show more sensitivity to pH and ionic strength during build-up. Due to the effect of aggregation of PEM on PLGA, enzymatically degradable PEM on PLGA can also be used to control the aggregate size of nanoparticles, which slowly disintegrates into its constituent particles with time, creating smaller size distribution after a period of time. This may provide control of penetrability into the cell.

References

- [1] Anderson, J. and Shive, M. *Advanced Drug Delivery Reviews* **28**(1), 5–24 OCT 13 (1997). [1](#), [8](#)
- [2] Okada, H. and Toguchi, H. *Critical Reviews in Therapeutic Drug Carrier Systems* **12**(1), 1–99 (1995). [1](#)
- [3] Jain, R. A. *Biomaterials* **21**(23), 2475–2490 (2000). [1](#), [9](#)
- [4] Abbas, A. O., Donovan, M. D., and Salem, A. K. *Journal of Pharmaceutical Sciences* **97**(7), 2448–2461 (2008). [1](#)
- [5] Jiang, W. L., Gupta, R. K., Deshpande, M. C., and Schwendeman, S. P. *Advanced Drug Delivery Reviews* **57**(3), 391–410 (2005). [1](#)
- [6] van der Walle, C. F., Sharma, G., and Kumar, M. *Expert Opinion on Drug Delivery* **6**(2), 177–186 (2009). [1](#)
- [7] Stevanovic, M. and Uskokovic, D. *Current Nanoscience* **5**(1), 1–14 (2009). [1](#)
- [8] Acharya, S. and Sahoo, S. K. *Advanced Drug Delivery Reviews* **63**(3), 170–183 (2011). [1](#), [20](#)
- [9] Wischke, C. and Schwendeman, S. P. *International Journal of Pharmaceutics* **364**(2), 298–327 (2008). [1](#)
- [10] Allison, S. D. *Expert Opinion on Drug Delivery* **5**(6), 615–628 (2008). [1](#), [11](#)
- [11] Huang, X. and Brazel, C. S. *Journal of Controlled Release* **73**(2-3), 121–136 (2001). [1](#), [11](#)
- [12] De Geest, B. G., Sukhorukov, G. B., and Mohwald, H. *Expert Opinion on Drug Delivery* **6**(6), 613–624 (2009). [2](#)
- [13] Zhou, J., Moya, S., Ma, L., Gao, C. Y., and Shen, J. C. *Macromolecular Bioscience* **9**(4), 326–335 (2009). [2](#), [19](#), [20](#), [21](#), [22](#), [23](#)
- [14] Chandy, T., Das, G. S., and Rao, G. H. R. *Journal of Microencapsulation* **17**(5), 625–638 (2000). [2](#)

-
- [15] Kawashima, Y., Yamamoto, H., Takeuchi, H., and Kuno, Y. *Pharmaceutical Development and Technology* **5**(1), 77–85 (2000). [2](#)
- [16] Liu, D. Z., Chen, W. P., Lee, C. P., Wu, S. L., Wang, Y. C., and Chung, T. W. *Journal of Microencapsulation* **21**(6), 643–652 (2004). [2](#)
- [17] Zheng, C. H., Gao, J. Q., Zhang, Y. P., and Liang, W. Q. *Biochemical and Biophysical Research Communications* **323**(4), 1321–1327 (2004). [2](#), [73](#)
- [18] Fischer, S., Foerg, C., Ellenberger, S., Merkle, H. P., and Gander, B. *Journal of Controlled Release* **111**(1-2), 135–144 (2006). [2](#)
- [19] Fischer, S., Uetz-von Allmen, E., Waeckerle-Men, Y., Groettrup, M., Merkle, H. P., and Gander, B. *Biomaterials* **28**(6), 994–1004 (2007). [2](#)
- [20] Na, K., Kim, S., Park, K., Kim, K., Woo, D. G., Kwon, I. C., Chung, H. M., and Park, K. H. *Journal of the American Chemical Society* **129**(18), 5788 (2007). [2](#)
- [21] Nafee, N., Taetz, S., Schneider, M., Schaefer, U. F., and Lehr, C. M. *Nanomedicine-Nanotechnology Biology and Medicine* **3**(3), 173–183 (2007). [2](#)
- [22] Manca, M. L., Loy, G., Zaru, M., Fadda, A. M., and Antimisiaris, S. G. *Colloids and Surfaces B-Biointerfaces* **67**(2), 166–170 (2008). [2](#)
- [23] Tahara, K., Sakai, T., Yamamoto, H., Takeuchi, H., and Kawashima, Y. *International Journal of Pharmaceutics* **354**(1-2), 210–216 (2008). [2](#)
- [24] Kakade, S., Manickam, D. S., Handa, H., Mao, G. Z., and Oupicky, D. *International Journal of Pharmaceutics* **365**(1-2), 44–52 (2009). [2](#)
- [25] Yang, Y. W. and Hsu, P. Y. J. *Biomaterials* **29**(16), 2516–2526 (2008). [2](#)
- [26] Zhou, J., Romero, G., Rojas, E., Ma, L., Moya, S., and Gao, C. Y. *Journal of Colloid and Interface Science* **345**(2), 241–247 (2010). [2](#)
- [27] Singh, V. and Tiwari, M. *International Journal of Polymer Science* (2010). [7](#)
- [28] Gilding, D. K. and Reed, A. M. *Polymer* **20**(12), 1459–1464 (1979). [7](#)
- [29] Baker, R. W. *Controlled Release of Biologically Active Agents*. Wiley & Sons, New York, (1987). [7](#)
- [30] Burkersroda, F. v., Schedl, L., and Göpferich, A. *Biomaterials* **23**(21), 4221–4231 (2002). [8](#)
- [31] Alexis, F. *Polymer International* **54**(1), 36–46 (2005). [8](#)
- [32] Holy, C. E., Dang, S. M., Davies, J. E., and Shoichet, M. S. *Biomaterials* **20**(13), 1177–1185 (1999). [8](#)

REFERENCES

- [33] Zolnik, B. S. and Burgess, D. J. *Journal of Controlled Release* **122**(3), 338–344 (2007). [8](#)
- [34] Park, T. G. *Biomaterials* **16**(15), 1123–1130 oct (1995). [8](#)
- [35] Vert, M., Mauduit, J., and Li, S. M. *Biomaterials* **15**(15), 1209–1213 dec (1994). [8](#)
- [36] Li, S. M. *Journal of Biomedical Materials Research* **48**(3), 342–353 June (1999). [8](#)
- [37] Alexis, F., Venkatraman, S., Rath, S. K., and Gan, L. H. *Journal of Applied Polymer Science* **102**(4), 3111–3117 (2006). [8](#)
- [38] Klose, D., Siepmann, F., Elkharraz, K., Krenzlin, S., and Siepmann, J. *International Journal of Pharmaceutics* **314**(2), 198–206 (2006). [8](#), [11](#)
- [39] Freiberg, S. and Zhu, X. X. *International Journal of Pharmaceutics* **282**(1-2), 1–18 (2004). [9](#), [10](#), [11](#)
- [40] Odonnell, P. B. and McGinity, J. W. *Advanced Drug Delivery Reviews* **28**(1), 25–42 (1997). [9](#), [22](#)
- [41] Crotts, G. and Park, T. G. *Journal of Controlled Release* **35**(2-3), 91–105 (1995). [10](#)
- [42] Jeyanthi, R., Thanoo, B. C., Metha, R. C., and DeLuca, P. P. *Journal of Controlled Release* **38**(2-3), 235–244 (1996). [10](#)
- [43] Ghaderi, R., Stureson, C., and Carlfors, J. *International Journal of Pharmaceutics* **141**(1-2), 205–216 (1996). [10](#)
- [44] Yang, Y. Y., Chung, T. S., Bai, X. L., and Chan, W. K. *Chemical Engineering Science* **55**(12), 2223–2236 (2000). [10](#)
- [45] McGee, J. P., Singh, M., Li, X. M., Qiu, H., and Ohagan, D. T. *Journal of Microencapsulation* **14**(2), 197–210 (1997). [10](#)
- [46] Jalil, R. and Nixon, J. R. *Journal of Microencapsulation* **7**(1), 25–39 (1990). [10](#)
- [47] Chen, J. L., Yeh, M. K., and Chiang, C. H. *Journal of Microencapsulation* **21**(8), 877–888 (2004). [10](#), [73](#)
- [48] Fredenberg, S., Wahlgren, M., Reslow, M., and Axelsson, A. *International Journal of Pharmaceutics* **415**(1-2), 34–52 (2011). [11](#)
- [49] Luan, X. S. and Bodmeier, R. *European Journal of Pharmaceutics and Biopharmaceutics* **63**(2), 205–214 (2006). [11](#)
- [50] Kang, J., Lambert, O., Ausborn, M., and Schwendeman, S. P. *International Journal of Pharmaceutics* **357**(1-2), 235–243 (2008). [11](#)

-
- [51] Blanco, M. D. and Alonso, M. J. *European Journal of Pharmaceutics and Biopharmaceutics* **43**(3), 287–294 (1997). [11](#)
- [52] Kang, J. C. and Schwendeman, S. P. *Molecular Pharmaceutics* **4**(1), 104–118 (2007). [11](#)
- [53] Xu, Q. G. and Czemuszka, J. I. *Journal of Controlled Release* **127**(2), 146–153 (2008). [11](#)
- [54] Faisant, N., Akiki, J., Siepmann, F., Benoit, J. P., and Siepmann, J. *International Journal of Pharmaceutics* **314**(2), 189–197 (2006). [11](#), [12](#)
- [55] Makino, K., Mogi, T., Ohtake, N., Yoshida, M., Ando, S., Nakajima, T., and Ohshima, H. *Colloids and Surfaces B: Biointerfaces* **19**(2), 173–179 (2000). [11](#)
- [56] Siepmann, J., Faisant, N., Akiki, J., Richard, J., and Benoit, J. P. *Journal of Controlled Release* **96**(1), 123–134 (2004). [11](#), [12](#)
- [57] Thombre, A. G. and Himmelstein, K. J. *Aiche Journal* **31**(5), 759–766 (1985). [12](#)
- [58] Baker, R. *Controlled Release of Biologically Active Agents*. Wiley, (1987). [12](#)
- [59] Harland, R. S., Dubernet, C., Benoit, J. P., and Peppas, N. A. *Journal of Controlled Release* **7**(3), 207–215 (1988). [12](#)
- [60] Charlier, A., Leclerc, B., and Couarraze, G. *International Journal of Pharmaceutics* **200**(1), 115–120 (2000). [12](#)
- [61] Raman, C., Berkland, C., Kim, K., and Pack, D. W. *Journal of Controlled Release* **103**(1), 149–158 (2005). [12](#)
- [62] Ehtezazi, T. and Washington, C. *Journal of Controlled Release* **68**(3), 361–372 (2000). [12](#), [13](#)
- [63] Batycky, R. P., Hanes, J., Langer, R., and Edwards, D. A. *Journal of Pharmaceutical Sciences* **86**(12), 1464–1477 (1997). [12](#)
- [64] He, J. T., Zhong, C. L., and Mi, J. G. *Drug Delivery* **12**(5), 251–259 (2005). [12](#)
- [65] Rothstein, S. N., Federspiel, W. J., and Little, S. R. *Journal of Materials Chemistry* **18**(16), 1873–1880 (2008). [12](#), [13](#)
- [66] Allison, S. D. *Journal of Pharmaceutical Sciences* **97**(6), 2024–2037 (2008). [12](#)
- [67] Lao, L. L., Venkatraman, S. S., and Peppas, N. A. *European Journal of Pharmaceutics and Biopharmaceutics* **70**(3), 796–803 (2008). [12](#)
- [68] Schonhoff, M. *Journal of Physics-Condensed Matter* **15**(49), R1781–R1808 (2003). [13](#)

REFERENCES

- [69] Sukhishvili, S. A. *Current Opinion in Colloid & Interface Science* **10**(1-2), 37–44 (2005). [13](#)
- [70] Boudou, T., Crouzier, T., Ren, K. F., Blin, G., and Picart, C. *Advanced Materials* **22**(4), 441–467 (2010). [13](#)
- [71] del Mercato, L. L., Rivera-Gil, P., Abbasi, A. Z., Ochs, M., Ganas, C., Zins, I., Sonnichsen, C., and Parak, W. J. *Nanoscale* **2**(4), 458–467 (2010). [13](#)
- [72] Hammond, P. T. *Advanced Materials* **16**(15), 1271–1293 (2004). [14](#)
- [73] Mjahed, H., Voegel, J. C., Chassepot, A., Senger, B., Schaaf, P., Boulmedais, F., and Ball, V. *Journal of Colloid and Interface Science* **346**(1), 163–171 (2010). [15](#)
- [74] Fery, A., Scholer, B., Cassagneau, T., and Caruso, F. *Langmuir* **17**(13), 3779–3783 (2001). [15](#)
- [75] Shchukin, D. G., Gorin, D. A., and Moehwald, H. *Langmuir* **22**(17), 7400–7404 (2006). [15](#)
- [76] Lavalle, P., Gergely, C., Cuisinier, F. J. G., Decher, G., Schaaf, P., Voegel, J. C., and Picart, C. *Macromolecules* **35**(11), 4458–4465 (2002). [15](#), [17](#)
- [77] Laugel, N., Betscha, C., Winterhalter, M., Voegel, J. C., Schaaf, P., and Ball, V. *Journal of Physical Chemistry B* **110**(39), 19443–19449 (2006). [15](#)
- [78] Hubsch, E., Ball, V., Senger, B., Decher, G., Voegel, J. C., and Schaaf, P. *Langmuir* **20**(5), 1980–1985 (2004). [15](#)
- [79] Ruths, J., Essler, F., Decher, G., and Riegler, H. *Langmuir* **16**(23), 8871–8878 (2000). [15](#), [16](#), [94](#)
- [80] Salomaki, M., Vinokurov, I. A., and Kankare, J. *Langmuir* **21**(24), 11232–11240 (2005). [15](#), [51](#)
- [81] Buscher, K., Graf, K., Ahrens, H., and Helm, C. A. *Langmuir* **18**(9), 3585–3591 (2002). [16](#)
- [82] Ladam, G., Schaad, P., Voegel, J. C., Schaaf, P., Decher, G., and Cuisinier, F. *Langmuir* **16**(3), 1249–1255 (2000). [16](#), [39](#), [52](#)
- [83] Steitz, R., Leiner, V., Siebrecht, R., and von Klitzing, R. *Colloids and Surfaces A-Physicochemical and Engineering Aspects* **163**(1), 63–70 (2000). [16](#)
- [84] McAloney, R. A., Sinyor, M., Dudnik, V., and Goh, M. C. *Langmuir* **17**(21), 6655–6663 (2001). [16](#), [17](#), [31](#)
- [85] Glinel, K., Moussa, A., Jonas, A. M., and Laschewsky, A. *Langmuir* **18**(4), 1408–1412 (2002). [16](#)

-
- [86] Yoo, D., Shiratori, S. S., and Rubner, M. F. *Macromolecules* **31**(13), 4309–4318 (1998). [16](#), [31](#)
- [87] Fujii, N., Fujimoto, K., Michinobu, T., Akada, M., Hill, J. P., Shiratori, S., Ariga, K., and Shigehara, K. *Macromolecules* **43**(8), 3947–3955 (2010). [16](#)
- [88] Elzieciak, A., Zapotoczny, S., Nowak, P., Krastev, R., Nowakowska, M., and Warszynski, P. *Langmuir* **25**(5), 3255–3259 (2009). [16](#)
- [89] Shiratori, S. S. and Rubner, M. F. *Macromolecules* **33**(11), 4213–4219 (2000). [16](#)
- [90] Bosio, V., Dubreuil, F., Bogdanovic, G., and Fery, A. *Colloids and Surfaces A-Physicochemical and Engineering Aspects* **243**(1-3), 147–155 (2004). [16](#), [39](#), [52](#)
- [91] Antipov, A. A., Shchukin, D., Fedutik, Y., Petrov, A. I., Sukhorukov, G. B., and Mohwald, H. *Colloids and Surfaces A-Physicochemical and Engineering Aspects* **224**(1-3), 175–183 (2003). [16](#)
- [92] Tong, W. J., Dong, W. F., Gao, C. Y., and Mohwald, H. *Journal of Physical Chemistry B* **109**(27), 13159–13165 (2005). [16](#), [52](#)
- [93] Decher, G. *Science* **277**(5330), 1232–1237 (1997). [16](#)
- [94] Arys, X., Laschewsky, A., and Jonas, A. M. *Macromolecules* **34**(10), 3318–3330 (2001). [16](#)
- [95] Muthukumar, M. *Journal of Chemical Physics* **86**(12), 7230–7235 (1987). [17](#)
- [96] Vandesteeg, H. G. M., Stuart, M. A. C., Dekeizer, A., and Bijsterbosch, B. H. *Langmuir* **8**(10), 2538–2546 (1992). [17](#)
- [97] Dobrynin, A. V., Colby, R. H., and Rubinstein, M. *Macromolecules* **28**(6), 1859–1871 (1995). [17](#)
- [98] Nazaran, P., Bosio, V., Jaeger, W., Anghel, D. F., and von Klitzing, R. *Journal of Physical Chemistry B* **111**(29), 8572–8581 (2007). [17](#)
- [99] Stanton, B. W., Harris, J. J., Miller, M. D., and Bruening, M. L. *Langmuir* **19**(17), 7038–7042 (2003). [17](#)
- [100] Zhu, Z. Q., Feng, X. S., and Penlidis, A. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* **26**(1), 1–8 (2006). [17](#)
- [101] Park, M. K., Deng, S. X., and Advincula, R. C. *Journal of the American Chemical Society* **126**(42), 13723–13731 (2004). [17](#)
- [102] Miller, M. D. and Bruening, M. L. *Chemistry of Materials* **17**(21), 5375–5381 (2005). [17](#)

REFERENCES

- [103] Calvo, E. J. and Wolosiuk, A. *Journal of the American Chemical Society* **124**(28), 8490–8497 (2002). [17](#)
- [104] Sullivan, D. M. and Bruening, M. L. *Chemistry of Materials* **15**(1), 281–287 (2003). [17](#)
- [105] Mansouri, S., Winnik, F. M., and Tabrizian, M. *Expert Opinion on Drug Delivery* **6**(6), 585–597 (2009). [18](#)
- [106] Harris, J. J. and Bruening, M. L. *Langmuir* **16**(4), 2006–2013 (2000). [18](#), [51](#), [54](#)
- [107] VonKlitzing, R. and Mohwald, H. *Macromolecules* **29**(21), 6901–6906 (1996). [18](#)
- [108] Antipov, A. A., Sukhorukov, G. B., and Mohwald, H. *Langmuir* **19**(6), 2444–2448 (2003). [18](#), [22](#), [43](#), [48](#)
- [109] Qiu, X. P., Donath, E., and Mohwald, H. *Macromolecular Materials and Engineering* **286**(10), 591–597 (2001). [18](#), [19](#), [43](#), [48](#)
- [110] Antipov, A. A., Sukhorukov, G. B., Donath, E., and Mohwald, H. *Journal of Physical Chemistry B* **105**(12), 2281–2284 (2001). [18](#), [19](#), [43](#), [48](#), [51](#), [58](#)
- [111] Ai, H., Jones, S. A., de Villiers, M. M., and Lvov, Y. M. *Journal of Controlled Release* **86**(1), 59–68 (2003). [19](#)
- [112] Pargaonkar, N., Lvov, Y. M., Li, N., Steenekamp, J. H., and de Villiers, M. M. *Pharmaceutical Research* **22**(5), 826–835 (2005). [19](#)
- [113] Haidar, Z. S., Hamdy, R. C., and Tabrizian, M. *Biomaterials* **29**(9), 1207–1215 (2008). [19](#), [21](#), [102](#)
- [114] Ibarz, G., Dahne, L., Donath, E., and Mohwald, H. *Chemistry of Materials* **14**(10), 4059–4062 (2002). [19](#), [48](#)
- [115] Sukhorukov, G. B., Antipov, A. A., Voigt, A., Donath, E., and Mohwald, H. *Macromolecular Rapid Communications* **22**(1), 44–46 (2001). [19](#), [81](#)
- [116] Antipov, A. A. and Sukhorukov, G. B. *Advances in Colloid and Interface Science* **111**(1-2), 49–61 (2004). [19](#)
- [117] Grama, C. N., Ankola, D. D., and Kumar, M. *Current Opinion in Colloid & Interface Science* **16**(3), 238–245 (2011). [19](#), [20](#)
- [118] Prabha, S., Zhou, W.-Z., Panyam, J., and Labhasetwar, V. *International Journal of Pharmaceutics* **244**(1-2), 105–115 (2002). [19](#)
- [119] Panyam, J. and Labhasetwar, V. *Advanced Drug Delivery Reviews* **55**(3), 329 – 347 (2003). [19](#), [20](#), [73](#)
- [120] Win, K. Y. and Feng, S.-S. *Biomaterials* **26**(15), 2713 – 2722 (2005). [19](#)

-
- [121] Desai, M. P., Labhasetwar, V., Walter, E., Levy, R. J., and Amidon, G. L. *Pharmaceutical Research* **14**(11), 1568–1573 (1997). [19](#)
- [122] Desai, M., Labhasetwar, V., Amidon, G., and Levy, R. *Pharmaceutical Research* **13**(12), 1838–1845 (1996). [19](#)
- [123] Qaddoumi, M. G., Ueda, H., Yang, J., Davda, J., Labhasetwar, V., and Lee, V. H. *Pharmaceutical Research* **21**(4), 641–648 (2004). [19](#)
- [124] Patel, N. R., Damann, K., Leonardi, C., and Sabliov, C. M. *Nanomedicine* **6**(8), 1381–1395 (2011). [19](#)
- [125] Danhier, F., Ansorena, E., Silva, J. M., Coco, R., Le Breton, A., and Preat, V. *Journal of Controlled Release* **161**(2), 505–522 (2012). [20](#)
- [126] Hamdy, S., Haddadi, A., Hung, R. W., and Lavasanifar, A. *Advanced Drug Delivery Reviews* **63**(10-11), 943–955 (2011). [20](#)
- [127] Zhang, X. Y., Sun, M. Z., Zheng, A. P., Cao, D. Y., Bi, Y. Q., and Sun, J. X. *European Journal of Pharmaceutical Sciences* **45**(5), 632–638 (2012). [20](#), [21](#), [23](#)
- [128] Luo, R. C., Neu, B., and Venkatraman, S. S. *Small* **8**(16), 2585–2594 (2012). [20](#), [21](#), [22](#), [35](#)
- [129] Bordi, F., Sennato, S., and Truzzolillo, D. *Journal of Physics-Condensed Matter* **21**(20), 26 (2009). [20](#), [92](#)
- [130] Ruysschaert, T., Germain, M., Gomes, J., Fournier, D., Sukhorukov, G. B., Meier, W., and Winterhalter, M. *IEEE Transactions on Nanobioscience* **3**(1), 49–55 (2004). [20](#)
- [131] de Villiers, M. M., Otto, D. P., Strydom, S. J., and Lvov, Y. M. *Advanced Drug Delivery Reviews* **63**(9), 701–715 (2011). [20](#)
- [132] Jain, P., Jain, S., Prasad, K. N., Jain, S. K., and Vyas, S. P. *Molecular Pharmaceutics* **6**(2), 593–603 (2009). [21](#), [23](#)
- [133] Jain, S., Patil, S. R., Swarnakar, N. K., and Agrawal, A. K. *Molecular Pharmaceutics* **9**(9), 2626–2635 (2012). [21](#), [23](#), [90](#)
- [134] Volodkin, D., Mohwald, H., Voegel, J. C., and Ball, V. *Journal of Controlled Release* **117**(1), 111–120 (2007). [21](#), [22](#), [23](#)
- [135] Fujimoto, K., Toyoda, T., and Fukui, Y. *Macromolecules* **40**(14), 5122–5128 (2007). [21](#), [23](#)
- [136] Jain, S., Kumar, D., Swarnakar, N. K., and Thanki, K. *Biomaterials* **33**(28), 6758–6768 (2012). [21](#), [23](#), [49](#), [90](#)

REFERENCES

- [137] Tan, J. P. K., Wang, Q., and Tam, K. C. *Journal of Controlled Release* **128**(3), 248–254 (2008). [21](#), [23](#)
- [138] Ye, S. Q., Wang, C. Y., Liu, X. X., and Tong, Z. *Journal of Biomaterials Science-Polymer Edition* **16**(7), 909–923 (2005). [21](#)
- [139] Shu, S. J., Sun, C. Y., Zhang, X. G., Wu, Z. M., Wang, Z., and Li, C. X. *Acta Biomaterialia* **6**(1), 210–217 (2010). [21](#), [23](#), [77](#)
- [140] Agrawal, R., Shanavas, A., Yadav, S., Aslam, M., Bahadur, D., and Srivastava, R. *Journal of Biomedical Nanotechnology* **8**(1), 19–28 (2012). [22](#)
- [141] Yushu, H. and Venkatraman, S. *Journal of Applied Polymer Science* **101**(5), 3053–3061 (2006). [26](#)
- [142] Mukherjee, B., Santra, K., Pattnaik, G., and Ghosh, S. *International Journal of Nanomedicine* **3**(4), 487–496 (2008). [26](#)
- [143] Leo, E., Scatturin, A., Vighi, E., and Dalpiaz, A. *Journal of Nanoscience and Nanotechnology* **6**(9-10), 3070–3079 (2006). [26](#)
- [144] Graves, R. A., Moiseyev, R., Freeman, T., and Mandal, T. K. *Journal of Biomaterials Science-Polymer Edition* **16**(5), 585–596 (2005). [26](#)
- [145] Jiang, G., Thanoo, B. C., and DeLuca, P. P. *Pharmaceutical Development and Technology* **7**(4), 391–399 (2002). [26](#)
- [146] Boury, F., Marchais, H., Proust, J. E., and Benoit, J. P. *Journal of Controlled Release* **45**(1), 75–86 (1997). [26](#)
- [147] Lobo, R., Pereira-da Silva, M., Raposo, M., Faria, R., and Oliveira, O. *Nanotechnology* **10**(4), 389–393 (1999). [31](#)
- [148] Vonkilitzing, R. and Mohwald, H. *Langmuir* **11**(9), 3554–3559 (1995). [32](#)
- [149] Sahoo, S., Panyam, J., Prabha, S., and Labhasetwar, V. *Journal of Controlled Release* **82**(1), 105–114 (2002). [35](#), [73](#)
- [150] Stolnik, S., Garnett, M. C., Davies, M. C., Illum, L., Bousta, M., Vert, M., and Davis, S. S. *Colloids and Surfaces A-Physicochemical and Engineering Aspects* **97**(3), 235–245 (1995). [35](#)
- [151] Wright, K. M., Horobin, R. W., and Oparka, K. J. *Journal of Experimental Botany* **47**(304), 1779–1787 (1996). [42](#)
- [152] Machatha, S. G. and Yalkowsky, S. H. *International Journal of Pharmaceutics* **294**(1-2), 185–192 (2005). [42](#)
- [153] An, Z. H., Lu, G., Mohwald, H., and Li, J. B. *Chemistry-A European Journal* **10**(22), 5848–5852 (2004). [43](#), [48](#)

-
- [154] Jalil, R. and Nixon, J. R. *Journal of Microencapsulation* **7**(3), 297–325 (1990). [45](#)
- [155] Arshady, R. *Journal of Controlled Release* **17**(1), 1–21 (1991). [45](#)
- [156] Klose, D., Siepmann, F., Willart, J. F., Descamps, M., and Siepmann, J. *International Journal of Pharmaceutics* **383**(1-2), 123–131 (2010). [45](#), [47](#)
- [157] Klose, D., Siepmann, F., Elkhamz, K., and Siepmann, J. *International Journal of Pharmaceutics* **354**(1-2), 95–103 (2008). [47](#)
- [158] Fernandez-Carballido, A., Herrero-Vanrell, R., Molina-Martinez, I. T., and Pastoriza, P. *International Journal of Pharmaceutics* **279**(1-2), 33–41 (2004). [47](#)
- [159] Cheruvu, N. P. S., Escobar, E. R., and Kompella, U. B. *Investigative Ophthalmology & Visual Science* **46** (2005). [48](#)
- [160] Steele, T. W. J., Huang, C. L., Kumar, S., Widjaja, E., Boey, F. Y. C., Loo, J. S. C., and Venkatraman, S. S. *Journal of Pharmaceutical Sciences* **100**(10), 4317–4329 (2011). [49](#)
- [161] Richardson, J. J., Liang, K., Kempe, K., Ejima, H., Cui, J., and Caruso, F. *Advanced Materials* **25**(47), 6874–6878 (2013). [49](#)
- [162] Gao, C., Donath, E., Moya, S., Dudnik, V., and Mohwald, H. *European Physical Journal E* **5**(1), 21–27 (2001). [51](#)
- [163] Radtchenko, I. L., Sukhorukov, G. B., Leporatti, S., Khomutov, G. B., Donath, E., and Mohwald, H. *Journal of Colloid and Interface Science* **230**(2), 272–280 (2000). [51](#)
- [164] Leporatti, S., Gao, C., Voigt, A., Donath, E., and Mohwald, H. *European Physical Journal E* **5**(1), 13–20 (2001). [51](#)
- [165] Caruso, F., Niikura, K., Furlong, D. N., and Okahata, Y. *Langmuir* **13**(13), 3422–3426 (1997). [51](#)
- [166] Losche, M., Schmitt, J., Decher, G., Bouwman, W. G., and Kjaer, K. *Macromolecules* **31**(25), 8893–8906 (1998). [51](#)
- [167] Kim, T. H. and Park, T. G. *International Journal of Pharmaceutics* **271**(1-2), 207–214 (2004). [61](#), [68](#), [71](#)
- [168] Mao, S., Xu, J., Cai, C., Germershaus, O., Schaper, A., and Kissel, T. *International Journal of Pharmaceutics* **334**(1-2), 137–148 (2007). [66](#)
- [169] Cai, C. F., Mao, S. R., Germershaus, O., Schaper, A., Rytting, E., Chen, D. W., and Kissel, T. *Journal of Microencapsulation* **26**(4), 334–345 (2009). [66](#)
- [170] Feczko, T., Toth, J., Dosa, G., and Gyenis, J. *Chemical Engineering and Processing* **50**(8), 757–765 (2011). [73](#)

REFERENCES

- [171] Panyam, J., Dali, M. A., Sahoo, S. K., Ma, W. X., Chakravarthi, S. S., Amidon, G. L., Levy, R. J., and Labhasetwar, V. *Journal of Controlled Release* **92**(1-2), 173–187 (2003). [73](#), [77](#)
- [172] Asghar, W., Islam, M., Wadajkar, A. S., Wan, Y., Ilyas, A., Nguyen, K. T., and Iqbal, S. M. *IEEE Transactions on Nanotechnology* **11**(3), 546–553 (2012). [73](#)
- [173] Wischke, C. and Borchert, H. H. *Pharmazie* **61**(9), 770–774 (2006). [73](#)
- [174] Igartua, M., Hernandez, R., Esquisabel, A., Gascon, A., Calvo, M., and Pedraz, J. *International Journal of Pharmaceutics* **169**(1), 45–54 (1998). [73](#)
- [175] Fu, K., Griebenow, K., Hsieh, L., Klibanov, A. M., and Langer, R. *Journal of Controlled Release* **58**(3), 357 – 366 (1999). [73](#)
- [176] Estey, T., Kang, J., Schwendeman, S., and Carpenter, J. *Journal of Pharmaceutical Sciences* **95**(7), 1626–1639 (2006). [73](#)
- [177] Zhang, J. X. and Zhu, K. J. *Journal of Microencapsulation* **21**(7), 775–785 (2004). [73](#)
- [178] Zhang, J. X., Chen, D., Wang, S. J., and Zhu, K. J. *Journal of Microencapsulation* **22**(4), 413–422 (2005). [73](#)
- [179] Nugraha, C., Bora, M., and Venkatraman, S. S. *PLoS ONE* **9**(3), e92393 03 (2014). [75](#), [83](#)
- [180] Yang, Y. Y., Chung, T. S., and Ng, N. P. *Biomaterials* **22**(3), 231–241 (2001). [76](#)
- [181] Blanco, M. D., Sastre, R. L., Teijón, C., Olmo, R., and Teijón, J. M. *International Journal of Pharmaceutics* **326**(1-2), 139–147 (2006). [77](#)
- [182] Viswanathan, N. B., Patil, S. S., Pandit, J. K., Lele, A. K., Kulkarni, M. G., and Mashelkar, R. A. *Journal of Microencapsulation* **18**(6), 783–800 (2001). [77](#)
- [183] Gupta, G. K., Jain, V., and Mishra, P. R. *Aaps Pharmscitech* **12**(1), 344–353 (2011). [77](#), [78](#)
- [184] Anandhakumar, S., Nagaraja, V., and Raichur, A. M. *Colloids and Surfaces B-Biointerfaces* **78**(2), 266–274 (2010). [77](#), [80](#)
- [185] Zhao, Q. H. and Li, B. Y. *Nanomedicine-Nanotechnology Biology and Medicine* **4**(4), 302–310 (2008). [77](#), [78](#), [80](#)
- [186] Shu, S. J., Zhang, X. G., Wu, Z. M., Wang, Z., and Li, C. X. *Biomaterials* **31**(23), 6039–6049 (2010). [77](#)
- [187] Tripathy, J. and Raichur, A. M. *Colloids and Surfaces B-Biointerfaces* **101**, 487–492 (2013). [77](#)

- [188] Sato, K., Hoshina, S., and Anzai, J. *Polymer Bulletin* **68**(3), 891–900 (2012). [78](#), [80](#)
- [189] Ibarz, G., Dahne, L., Donath, E., and Mohwald, H. *Advanced Materials* **13**(17), 1324–1327 (2001). [81](#)
- [190] Sukhorukov, G. B., Brumen, M., Donath, E., and Mohwald, H. *Journal of Physical Chemistry B* **103**(31), 6434–6440 (1999). [82](#)
- [191] Song, C. X., Labhasetwar, V., Murphy, H., Qu, X., Humphrey, W. R., Shebuski, R. J., and Levy, R. J. *Journal of Controlled Release* **43**(2-3), 197–212 (1997). [83](#)
- [192] Cal, C. F., Bakowsky, U., Rytting, E., Schaper, A. K., and Kissel, T. *European Journal of Pharmaceutics and Biopharmaceutics* **69**(1), 31–42 (2008). [83](#)
- [193] Li, Y. P., Pei, Y. Y., Zhang, X. Y., Gu, Z. H., Zhou, Z. H., Yuan, W. F., Zhou, J. J., Zhu, J. H., and Gao, X. J. *Journal of Controlled Release* **71**(2), 203–211 (2001). [83](#)
- [194] Gebhart, B. *Heat Conduction and Mass Diffusion*. McGraw-Hill, (1993). [85](#)
- [195] Monge, C., Ren, K. F., Berton, K., Guillot, R., Peyrade, D., and Picart, C. *Tissue Engineering Part A* **18**(15-16), 1664–1676 (2012). [90](#)
- [196] Palama, I. E., D’Amone, S., Coluccia, A. M. L., and Gigli, G. *Biotechnology and Bioengineering* **110**(2), 586–596 (2013). [90](#)
- [197] Huang, X. B., Lin, N. M., Hang, R. Q., Wang, X. G., Zhang, X. Y., and Tang, B. *Carbohydrate Polymers* **92**(2), 1064–1070 (2013). [90](#)
- [198] Wang, Z. J., Zhu, H., Yang, W., Wang, X. L., Wang, J., and Yang, X. R. *Colloids and Surfaces A-Physicochemical and Engineering Aspects* **348**(1-3), 137–144 (2009). [90](#)
- [199] Pechenkin, M. A., Mohwald, H., and Volodkin, D. V. *Soft Matter* **8**(33), 8659–8665 (2012). [90](#)
- [200] Girod, S., Boissere, M., Longchambon, K., Begu, S., Tourne-Petheil, C., and Devoisselle, J. M. *Carbohydrate Polymers* **55**(1), 37–45 (2004). [90](#)
- [201] Sacco, D. and Dellacherie, E. *FEBS Letters* **199**(2), 254–258 (1986). [90](#)
- [202] Cuomo, F., Lopez, F., Ceglie, A., Maiuro, L., Miguel, M. G., and Lindman, B. *Soft Matter* **8**(16), 4415–4420 (2012). [90](#), [92](#)
- [203] Kato, N., Schuetz, P., Fery, A., and Caruso, F. *Macromolecules* **35**(26), 9780–9787 (2002). [90](#), [92](#)
- [204] Sennato, S., Truzzolillo, D., Bordi, F., Sciortino, F., and Cametti, C. *Colloids and Surfaces A-Physicochemical and Engineering Aspects* **343**(1-3), 34–42 (2009). [92](#)

REFERENCES

- [205] Keren, K., Soen, Y., Ben Yoseph, G., Gilad, R., Braun, E., Sivan, U., and Talmon, Y. *Physical Review Letters* **89**(8), 4 (2002). [92](#)
- [206] Gillies, G., Lin, W., and Borkovec, M. *Journal of Physical Chemistry B* **111**(29), 8626–8633 (2007). [92](#)
- [207] Ball, V., Winterhalter, M., Schwinte, P., Lavalley, P., Voegel, J. C., and Schaaf, P. *Journal of Physical Chemistry B* **106**(9), 2357–2364 (2002). [93](#)
- [208] Dejeu, J., Buisson, L., Guth, M. C., Roidor, C., Membrey, F., Charraut, D., and Foissy, A. *Colloids and Surfaces A-Physicochemical and Engineering Aspects* **288**(1-3), 26–35 (2006). [94](#)
- [209] Salis, A., Bostrom, M., Medda, L., Cugia, F., Barse, B., Parsons, D. F., Ninham, B. W., and Monduzzi, M. *Langmuir* **27**(18), 11597–11604 (2011). [97](#)
- [210] Crouzier, T. and Picart, C. *Biomacromolecules* **10**(2), 433–442 (2009). [102](#)
- [211] Kittitheeranun, P., Sanchavanakit, N., Sajomsang, W., and Dubas, S. T. *Langmuir* **26**(10), 6869–6873 (2010). [102](#)