

FUNCTIONAL THIN FILM COATINGS OF PORPHYRINS AND PHTHALOCYANINES BY LAYER-BY-LAYER ASSEMBLY

by

Yonca Belce

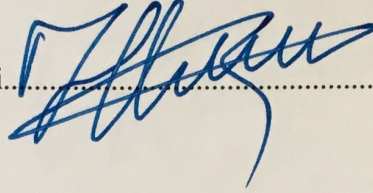
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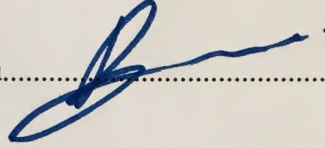
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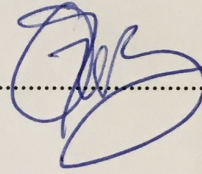
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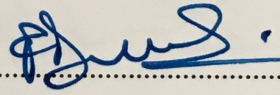
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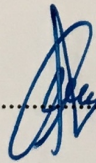
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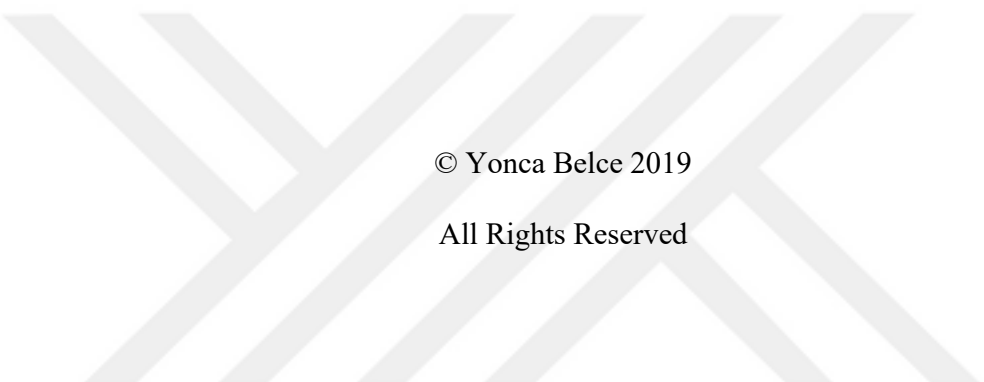
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ABSTRACT

FUNCTIONAL THIN FILM COATINGS OF PORPHYRINS AND PHTHALOCYANINES BY LAYER-BY-LAYER ASSEMBLY

YONCA BELCE

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Thesis Supervisor: Assoc. Prof. Fevzi Çakmak Cebeci

Keywords: Layer-by-Layer self-assembly, electrostatic interaction, multilayer, thin film coating, phthalocyanine, porphyrin, corrosion-protection, photodynamic therapy, nanosphere formation

Layer-by-layer self-assembly is a versatile and environmental-friendly deposition mechanism for functional thin film coatings. Aqueous dispersions of nanoparticles, polyelectrolytes and macrocyclic compounds are well-known molecular candidates for LbL deposition. Nevertheless, in order to have controlled film properties in nanometer scale pH, concentration, deposition architecture and material distribution need to be precisely adjusted. Prepared multilayer coatings by LbL mechanism can demonstrate exceptional improvements in various application fields such as corrosion-protection and photodynamic therapy.

Phthalocyanines and porphyrins are highly favored macrocyclic molecules in numerous areas due to their π -conjugated, delocalized electronic structures. Solar cells,

photodynamic therapy, liquid crystals are only some of the functional utilization areas of phthalocyanines. Although their hydrophobic feature is desired for practical purposes, attachment of hydrophilic functional groups make them great candidates for LbL deposition.

In this doctoral study, various metallated phthalocyanine types and their derivatives have been used for homogeneous and uniform thin film formation via layer-by-layer (LbL) coating method for potential anti-corrosive and photodynamic therapy purposes. Influence of pH and concentration for nickel(II)phthalocyanine tetrasulfonic acid tetrasodium salt on multilayer film properties; protection of alternating bilayer and tetralayer coatings of oppositely charged polyelectrolytes with nickel(II)phthalocyanine tetrasulfonic acid tetrasodium salt and copper phthalocyanine- 3,4',4'',4'''-tetrasulfonic acid tetrasodium salt against corrosion; surface distribution of encapsulated zinc(II) phthalocyanine nanospheres and finally sequential adsorption of 5,10,15,20-(tetra-4-carboxyphenyl)porphyrin and zinc(II) phthalocyanine tetrasulfonic acid are examined on glass, silicon wafer and stainless-steel substrates by LbL mechanism.

Dynamic light scattering (DLS) is applied for zeta potential determination. Electrochemical measurements are performed by potentiostat for anti-corrosion properties of multilayer films. Surface distribution of encapsulated nanospheres and topography are analyzed by scanning electron microscopy (SEM) and atomic force microscopy (AFM) respectively. Thickness of deposited coatings are evaluated by surface profiler and spectroscopic ellipsometry. In addition, quartz-crystal microbalance detector (QCM-D) is utilized for adsorption amount detection. Due to the colorful appearances of prepared coatings, multilayer film growth is monitored by ultra-violet visible spectroscopy (UV-Vis).

Overall, sequential adsorption of polyelectrolytes and different phthalocyanine-derivatives are successfully controlled. Obtained multilayer thin films are promising candidates for corrosion protection and photodynamic therapy.

ÖZET

PORFİRİN VE FİTALOSİYANİNLERİN TABAKA TABAKA KAPLAMA METODU İLE FONKSİYONEL İNCE FİMLERİNİN HAZIRLANMASI

YONCA BELCE

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Tez Danışmanı: Doç. Dr. Fevzi Çakmak Cebeci

Anahtar Kelimeler: tabaka tabaka kaplama, elektrostatik etkileşim, çoklu tabaka, ince film kaplama, fitalosiyanın, porfirin, korozyondan koruma, fotodinamik terapi, nanoküre yapımı

Tabaka tabaka kaplama (LbL) yöntemi fonksiyonel ince film kaplamaları için çok yönlü ve çevre dostu bir biriktirme yöntemidir. Nanoparçacıkların, polielektrolitlerin ve makro halkalı bileşiklerin sulu çözeltileri LbL yöntemi için bilinen en iyi molekül adayları arasındadır. Ancak nanometre ölçeğinde kontrollü film özelliklerine sahip olabilmek için pH, konsantrasyon, biriktirme yapısı ve malzeme dağılımı tam olarak ayarlanmalıdır. LbL mekanizmasıyla hazırlanan çok tabakalı kaplamalar korozyondan koruma ve fotodinamik terapi gibi çok çeşitli uygulama alanlarında istisnai gelişmeler gösterebilir.

Fitalosiyanın ve porfirinler π -konjuge ve delokalize elektron yapıları nedeniyle çok sayıda alanda tercih edilen makro halkalı moleküllerdir. Güneş pilleri, fotodinamik terapi,

sıvı kristaller fitalosiyaninlerin fonksiyonel uygulama alanlarından yalnızca bazılarıdır. Pratik amaçlar nedeniyle hidrofobik özellikleri tercih edilse de hidrofilik fonksiyonel grupların eklenmesiyle tabaka tabaka kaplama yöntemi için ideal adaylardır.

Bu doktora çalışmasında, çeşitli metallenmiş fitalosiyanin yapıları ve türevleri kullanılarak homojen ve düzenli ince film oluşumu tabaka tabaka kaplama yöntemiyle (LbL) potansiyel korozyon karşıtı ve fotodinamik terapi uygulamaları için çalışılmıştır. Nikel (II) fitalosiyanin tetrasülfonik asit tetrasodyum tuzu için pH ve konsantrasyonun çok tabakalı film özellikleri üzerindeki etkisi; nikel (II) fitalosiyanin tetrasülfonik asit tetrasodyum tuzu ve bakır fitalosiyanin-3,4',4'',4'''-tetrasülfonik asit tetrasodyum tuzunun zıt yüklü polielektrolitlerle korozyona karşı koruması birbirini izleyen çift tabakalı ve dört tabakalı kaplamaları; enkapsüle edilmiş çinko (II) fitalosiyanin nanokürelerinin yüzey dağılımı ve son olarak 5,10,15,20-(tetra-4-karboksifenil)porfirin ve çinko(II) fitalosiyanin tetrasülfonik asit yapılarının sıralı adsorpsiyonu LbL mekanizması ile cam, silikon yonga levha ve çelik yüzeyler üzerinde incelenmiştir.

Dinamik ışık saçılımı (DLS) zeta potansiyel tayini için kullanılmıştır. Çok tabakalı filmlerin korozyon karşıtı özellikleri için elektrokimyasal ölçümler potansiyostat ile sağlanmıştır. Enkapsüle edilmiş nanokürelerin yüzey dağılımı ve topografisi sırasıyla taramalı elektron mikroskobu (SEM) ve atomic kuvvet mikroskobisi (AFM) ile analiz edilmiştir. Biriktirilen filmlerin kalınlıkları yüzey profilometresi ve spektroskopik elipsometre ile değerlendirilmiştir. Buna ek olarak, adsorbe edilen malzeme miktarı kuvarz kristal mikroterazi dedektörü (QCM-D) ile tayin edilmiştir. Hazırlanan kaplamaların renkli görünümünden faydalanarak çok tabakalı filmlerin büyümesi mor ötesi-görünür ışık spektroskopisi (UV-Vis) ile gözlemlenmiştir.

Genel olarak polielektrolitlerin ve fitalosiyanin türevi yapıların sıralı adsorpsiyonu başarılı bir şekilde kontrol edilebilmektedir. Elde edilen çok tabakalı ince filmler korozyondan koruma ve fotodinamik terapi alanları için gelecek vadede adaylardır.



To my beloved half Bekir...

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LIST OF ABBREVIATIONS

AFM	Atomic Force Microscopy
bL	bi Layer
bPEI	Branched poly (ethyleneimine)
CuPcTS	Copper phthalocyanine-3,4',4'',4'''-tetrasulfonic acid tetrasodium salt
CV	Cyclic Voltammetry
DLS	Dynamic Light Scattering
EIS	Electrochemical Impedance Spectroscopy
ITO	Indium tin oxide
LB	Langmuir-Blodgett
LbL	Layer-by-Layer
NiPcTS	Nickel(II)phthalocyanine tetrasulfonic acid tetrasodium salt
PAA	Poly Acrylic Acid
PAH	Poly Allylamine Hydrochloride
PDAC	Poly (diallyl dimethyl ammonium chloride)
Pc	Phthalocyanine
PLGA	Poly (D, L-lactide-co-glycolide)
Por	Porphyrin
PVA	Polyvinyl alcohol
QCM	Quartz-Crystal Microbalance
SAM	Self-Assembled Monolayers
SEM	Scanning Electron Microscopy
SPS	Poly (Sodium 4-styrenesulfonate)
Tl	tetra layer
UV-Vis	Ultraviolet-visible
ZnPc	Zinc(II)phthalocyanine
ZnPcTS	Zinc(II)phthalocyanine tetrasulfonic acid

1. Introduction

Nano-engineering is still a developing concept in materials science. Especially surface engineering mechanisms gain attention of several researchers due to its wide range of applicability. Layer-by-layer (LbL) self-assembly is one of the well-known and improving thin film coating methods in literature. Due to its sequential adsorption mechanism, LbL method presents great advantages over other functional thin film mechanisms in the literature. While oppositely charged polyelectrolytes are mostly preferred for LbL deposition due to their electrostatic interactions, multilayer thin films of macrocyclic molecules such as phthalocyanines and porphyrins are also investigated. For precise control over thin film formation certain parameters such as pH and concentration need to be analyzed. Although water-soluble materials are easily applicable by LbL technique, there is still a need for improvement on LbL deposition mechanism of hydrophobic molecules, which frequently appear in the biomedical field. In addition to drug delivery, there are numerous application areas where LbL coating is favored. In this thesis, significance of concentration and pH of sulfonated nickel phthalocyanine, influence of phthalocyanine distribution among film layers for anti-corrosive surfaces, transportation of hydrophobic phthalocyanines by encapsulation for future skin cancer treatments via photodynamic therapy and thin film engineering of sulfonated zinc phthalocyanine with carboxylated porphyrin are studied by layer-by-layer deposition method.

1.1. Motivation

The scope of this thesis is to investigate thin film engineering of porphyrin and hydrophilic/ hydrophobic phthalocyanine integrated coatings for potential anti-corrosive and photodynamic therapy applications by layer-by-layer coating mechanism.

Functionalized phthalocyanines and porphyrin fulfill one of the primary requirements for LbL deposition, water-solubility. Hence, it is expected to form homogeneous and uniform films by these macrocyclic compounds. It is known in the literature that pH, concentration, molecular weight, temperature, etc. are critical factors affecting LbL film

properties. By adjusting pH and concentration of phthalocyanines film thickness, roughness features will be controllable and predictable.

Physical encapsulation of hydrophobic molecules by polymeric structures is well-known for drug delivery in the literature. Transformation of the entrapment technique by LbL mechanism will be promising for surface-based applications.

1.2. Novelty of this thesis

Controllable surface coatings of certain macrocyclic compounds like phthalocyanines and carboxylated porphyrin by layer-by-layer self-assembly mechanism for different application fields is the main purpose of this thesis. Besides, precise control over LbL coating of encapsulated active molecule is a novel study in the literature.

1.3. Roadmap of this thesis

- Influence of pH and concentration on LbL coating for hydrophilic nickel phthalocyanine is investigated.
- Thin film architecture of hydrophilic nickel and copper phthalocyanines for corrosion protection is studied by LbL deposition.
- Multilayer thin film formation of encapsulated hydrophobic zinc phthalocyanine is examined by LbL mechanism for further photodynamic therapy purposes.
- Layer-by-layer method is utilized to explore thin film engineering of sulfonated zinc phthalocyanine and carboxylated porphyrin.

2. Literature Survey

2.1. Functional Thin Film Mechanisms

Functionality of a material arises from the combination of individual properties of each component. Diversity in material features offer great benefit in terms of functionality.

In the history of multilayer films, three thin film formation technologies have been reported. Self-assembly monolayers (SAM), Langmuir-Blodgett (LB) and layer-by-layer assembly (LbL) methods are widely used.

Among surface modification methods self-assembled monolayers (SAM) has been known since 1980's. It is defined as the monomolecular adsorption of surfactant molecules on a solid surface¹ to form organic thin films².

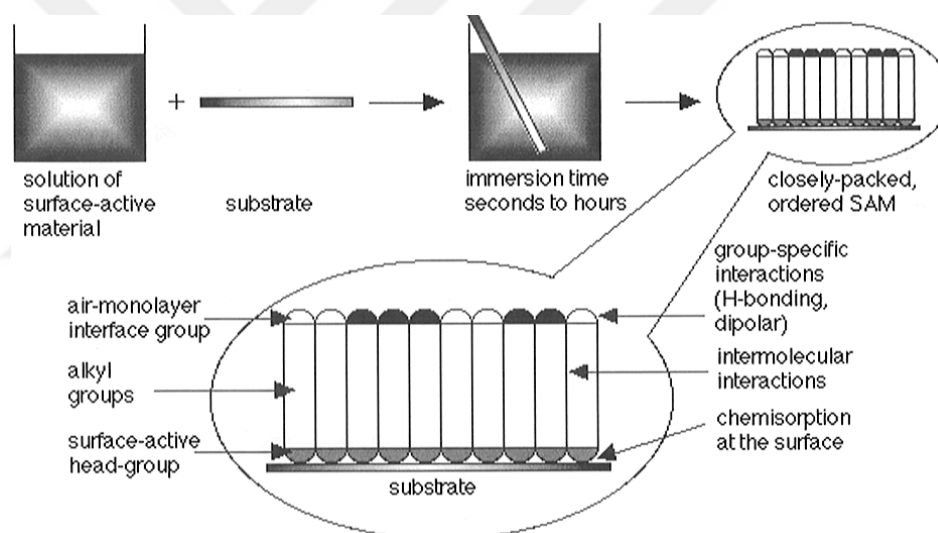


Figure 2.1. Formation mechanism of self-assembled monolayers (SAM) on a solid substrate¹

Langmuir-Blodgett (LB) technique is named after two researchers Irving Langmuir and Katharine Blodgett in early 1900's³. In order to form a single Langmuir monolayer a hydrophobic tail and a hydrophilic head is required. Typically, the hydrophilic part of the molecule consists of hydrogen bonding and polar functional groups⁴ such as -OH, -COOH or -NH₂. On the other hand, hydrophobic tail is usually made of a hydrocarbon chain. An air-water interface is necessary to build a distinct Langmuir monolayer.

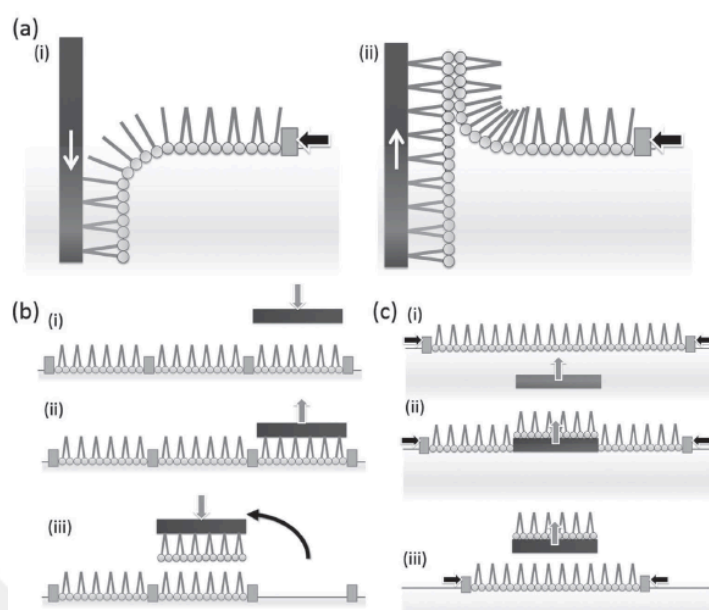


Figure 2.2. Thin film formation by⁵ (a) Langmuir-Blodgett (vertical dipping) technique (b) Langmuir-Schaefer (horizontal dipping) technique (c) horizontal drawing-up technique

Typically, a Langmuir trough is used for LB film fabrication. Experimentally, first the amphiphilic molecule is dissolved in an organic, volatile solvent such as chloroform, benzene or toluene. It is carefully placed onto an aqueous subphase, which is usually water, on a Langmuir trough⁴. As the organic solvent evaporates, amphiphilic molecule covers the surface of subphase. By using the limiting barriers on Langmuir trough, surface area and hence the surface pressure that an amphiphilic molecule occupy is controlled⁴. Surface area per molecule vs surface pressure gives the monolayer isotherm features. Langmuir-blodgett film is acquired once the monolayer is transferred to solid substrate that is usually silicon wafer, glass or quartz.

Hydrophilic-hydrophobic interactions between the substrate surface and monolayer are crucial for adsorption. Either hydrophobic substrate surface interacts with the hydrophobic tail of monolayer or the hydrophilic surface of the solid support with the hydrophilic head of amphiphilic molecule. Structural design and the thin film features⁶ (in nanometer scale) of Langmuir-Blodgett films provide various application areas. Optical applications⁴, sensors⁶, semiconductors⁵⁻⁶, solar cells⁶ are just few examples for LB films.

2.2. Layer-by-Layer Self-Assembly

Due to the limitations in Langmuir-Blodgett multilayer concept, layer-by-layer (LbL) deposition technique gain attention among multilayer thin film fabrication methods. LbL is a well-known, universal coating method since 1990s. It is an extensive method enabling deposition on various type of substrates, where water can access, with numerous substances such as biomolecules⁷⁻¹⁰, polymers¹¹⁻¹⁴, inorganic molecules¹⁵⁻¹⁷ or even nanoparticles¹⁸⁻¹⁹. Initiative studies require only electrostatic interaction²⁰⁻²¹ of oppositely charged molecules for layer-by-layer deposition. However, by the time of progress other type of interactions²⁰ like hydrogen bonding, biomolecular attachment or charge transfer for adsorption to underneath layer/ surface gain interest as well. Common LbL technologies involve immersion (dipping)²⁰, spraying^{20, 22} or spinning²⁰ the substrate to the coating material solution. Due to its versatile, gentle and practical features, LbL is appropriate for a wide range of applications. Not only in research but also in the industry layer-by-layer approach earn presence by its highly controlled architectural mechanism. Kim et.al.²³ proposed to use the thin film coating obtained by the LbL deposition of oppositely charged weak polyelectrolytes and TiO₂ for gas sensor applications. Similarly, according to the study of Fujita et. al.²⁴ anti-reflective thin films are fabricated by oppositely charged polyions. In addition to these, there are numerous examples of thin film coatings prepared by LbL self-assembly for fuel cell²⁵, biosensor²⁵, drug delivery²⁶, anti-UV²⁵, gene transfer²⁵ or biomedical²⁶ applications.

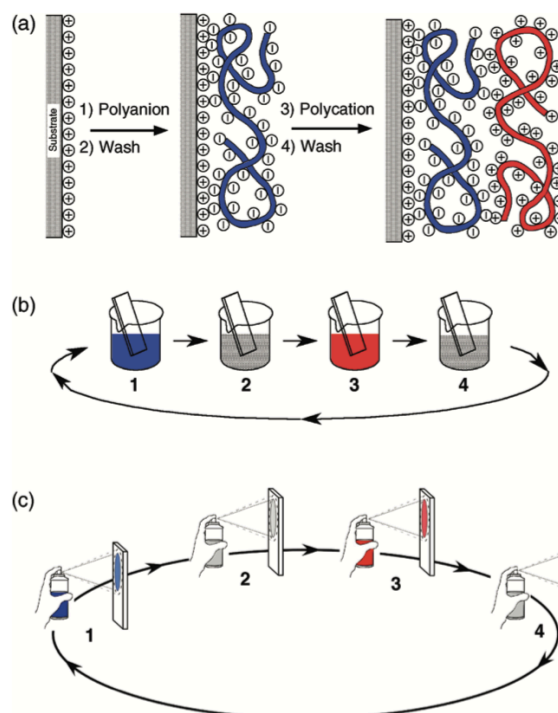


Figure 2.3. Layer-by-Layer deposition mechanism²⁷ (a) on substrate surface, (b) by dipping, (c) by spraying

The main mechanism of layer-by-layer self-assembly is initiated by the adsorption of a charged molecule to an oppositely charged substrate surface²⁷. Because of the repulsion of molecules with the same charge only a single layer on substrate surface is adsorbed. As given in [Figure 2.3](#) deposition mechanism carries on with the rinsing steps. In order to avoid cross-contamination, substrate surface needs to be rinsed such that the excess of the first adsorbing material is completely removed before the second adsorbing specie is introduced to the deposition process. Following that substrate is exposed to another molecule, nanoparticle or biomolecule with an opposite charge. Once the surface charge is reversed with the introduced layer rinsing steps are repeated for a uniform coating. This cycle is called as a “bilayer” and it is possible to repeat the process until desired thickness or number of layers are obtained. Traditionally dipping, spinning and spraying of substrate to the corresponding polyion solution are common LbL deposition mechanisms. In all techniques, a flat substrate surface is coated. However, it is not the only way to apply layer-by-layer deposition. Nowadays, encapsulation²⁸ over capsules²⁹ or nanoparticle²¹ cores is taking attention of many researchers, which is a promising tool for drug delivery. In order to build multilayer film, core material is dipped or spinned in a

coating material and rinsed with distilled water instead of a flat substrate. By sequential deposition of polyions, biomolecules or inorganic structures numbers of layers are coated on core material. In case of drug delivery, following the deposition process core material is decomposed in an appropriate environment by change in pH, temperature etc.

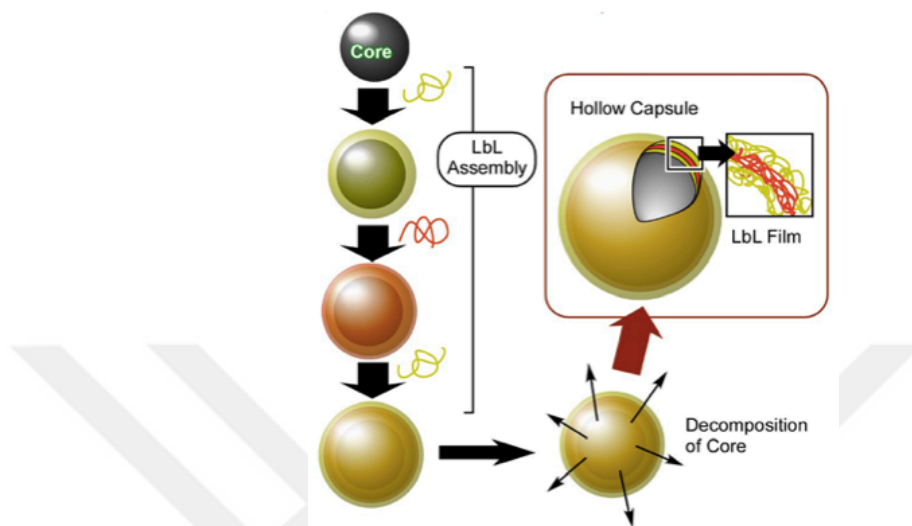


Figure 2.4. Encapsulation of core material via layer-by-layer method²⁹

Although LbL adsorption mechanism is based on electrostatic attractions of oppositely charged polyions, entropy is the primary driving force instead of enthalpy³⁰. Entropy of the system is enhanced when undissociated counterions are released upon interaction of charged substrate surface with a polyion³⁰. In addition to that, release of solvent structures from solvent shell of ionic groups reinforce entropy gain³⁰. Conversely, the number of electrostatic bonds does not alter for polyion adsorption in the entire system³⁰. Hence, variation in enthalpy is not significant. However, the number of ions per molecule adsorbed is critical³⁰.

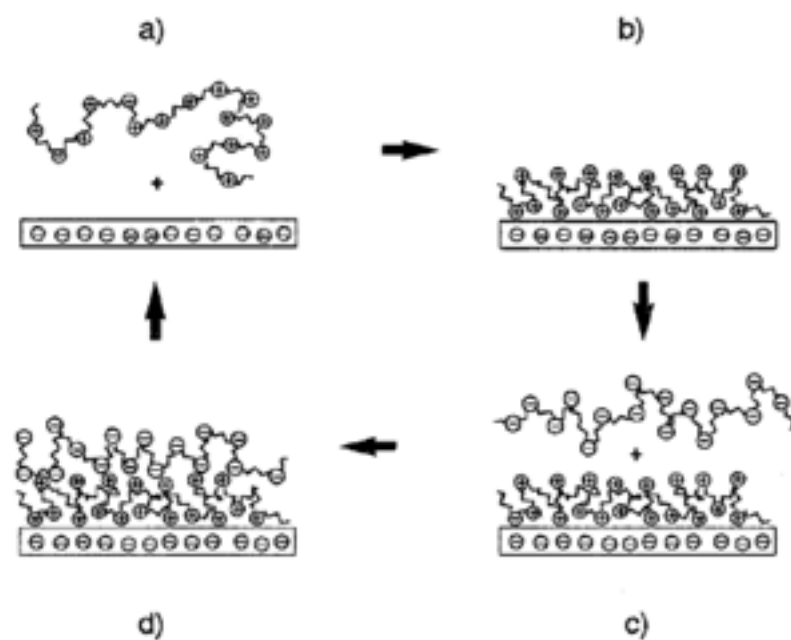


Figure 2.5. Schematic representation for layer-by-layer deposition by electrostatic interaction³⁰

Among several LbL mechanisms, dip-LbL is the most common technique due to its simplicity. It does not require any complex experimental setup. Coating material and wash bath containers are adequate to dip any si-wafer, glass, quartz or metal substrate. However, it is time consuming²⁰ when hundreds of layers are required. A single deposition step takes minimum 10 minutes and at least three wash baths are necessary. Approximately 30 min is the required experimental period for a single bilayer formation. Therefore, spray-LbL technique is preferred to reduce experimental process. Different from dip-coating method, only few seconds of spraying is enough to transfer the coating material to substrate surface. Compared to dip-LbL, spray method leads to thinner film formation^{20, 22}. Besides, spraying distance, spraying time and air pressure are some of the additional parameters^{20, 22} influencing thin film properties. Especially in the industry, spray-LbL has an advantage over other coating mechanisms due to its large-scale applicability²¹. On the other hand, the advantage of spin-LbL over other methods is the rapid solvent evaporation. It takes ~2-3min to deposit one bilayer on a flat surface. However, it is not possible to spin-coat 3D substrates. Furthermore, film thickness and uniformity are strongly dependent on angular speed²⁰ and viscosity²⁰ of coating materials. Beside traditional layer-by-layer coating techniques, numerous other mechanisms²¹ have been developed. Ink-jet printing, dip-pen lithography, electrodeposition and 3D bio-

printing are some of the other LbL technologies developed since 1990s²¹ and literature continues to develop new LbL fabrication procedures day-by-day.

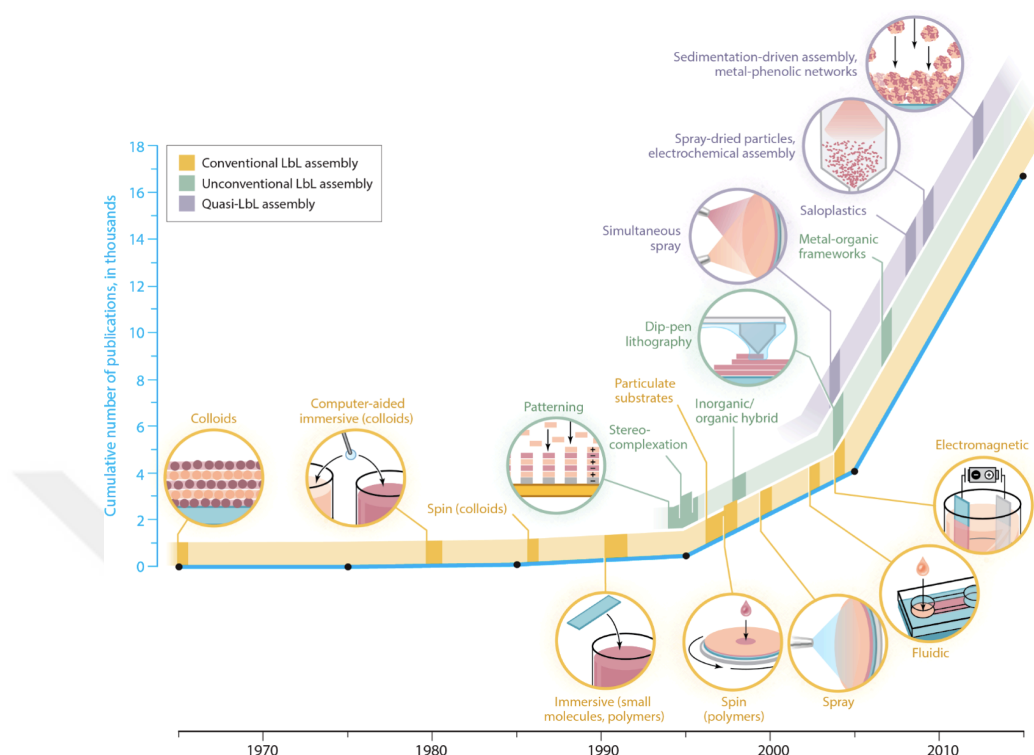


Figure 2.6. General progress of LbL mechanisms in the literature²¹

For a homogeneous and uniform coating there are few requirements to be considered for layer-by-layer deposition mechanism. First of all, LbL is a water-based system, which implies that all solvents for coating materials need to be water. Any organic solvent will contradict with the main LbL concept. In addition; as well as charge density³¹⁻³³(ionic concentration), temperature³³, deposition duration³¹ of adsorbing materials, speed and the number of adsorption²⁰ and rinsing periods are significant factors affecting the final thin film roughness, thickness and morphology. For a repeatable process it is essential to have control over these parameters.

Another key role on LbL approach is pH³⁴⁻³⁶, which strongly influences the ionic concentration. Especially for polyelectrolyte solutions it is vital to adjust pH value before any deposition process. Depending on the adjusted pH value, polyelectrolytes or any ionized specie changes its conformation in aqueous solution. Due to the conformational differences film thickness¹³ and roughness¹³ features are modified. Shiratori¹³ et. al. demonstrated that thin film properties of weak polyelectrolytes such as PAH (poly

allylamine hydrochloride) and PAA (polyacrylic acid) are totally dependent on their pH values. As given in [Figure 2.7](#), in the presence of high PAA pH (above 6.5) and low PAH pH (below 4.5) almost no film formation or ultra-thin film formation is accomplished¹³. Similarly, by controlling pH of deposited molecules it is achievable to form thicker films than 120Å. Besides, surface roughness is another pH-dependent outcome in LbL deposition mechanism. According to Shiratori¹³, between 6-7.5 pH surface roughness is ~10 Å, which is the consequence of fully ionized polyelectrolytes. Furthermore, fully ionized polyelectrolytes lead to highest thickness for the studied PAH-PAA film systems³⁷.

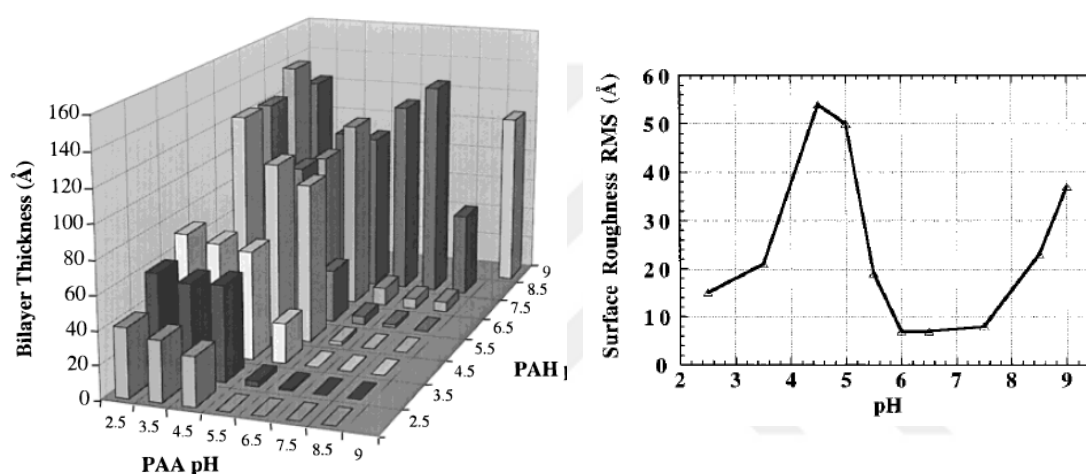


Figure 2.7. Effect of pH values¹³ on (a) film thickness (b) surface roughness

Especially for weak polyelectrolytes pH or charge density play a significant role on adsorption kinetics³⁷. While strong polyelectrolytes such as poly(diallyldimethyl ammonium chloride), PDAC or poly(vinyl sulfonic acid sodium salt), PVS are not influenced from pH changes, weak polyelectrolytes such as poly(allylamine hydrochloride), PAH or poly(acrylic acid (PAA) are strongly dependent on pH conditions, since electrostatic interactions are the primary driving force for layer-by-layer self-assembly formation.

Beside pH of the ionic solutions that are used to form nanofilms, concentration of the coating material or polyelectrolyte play a critical role as well. Elosua et. al.³⁸ demonstrated that thin film roughness and thickness are affected from the applied polyelectrolyte concentration values. As the concentration of polymeric solution

increases, corresponding RMS roughness and thickness values are higher than the films prepared with lower polymeric concentration.

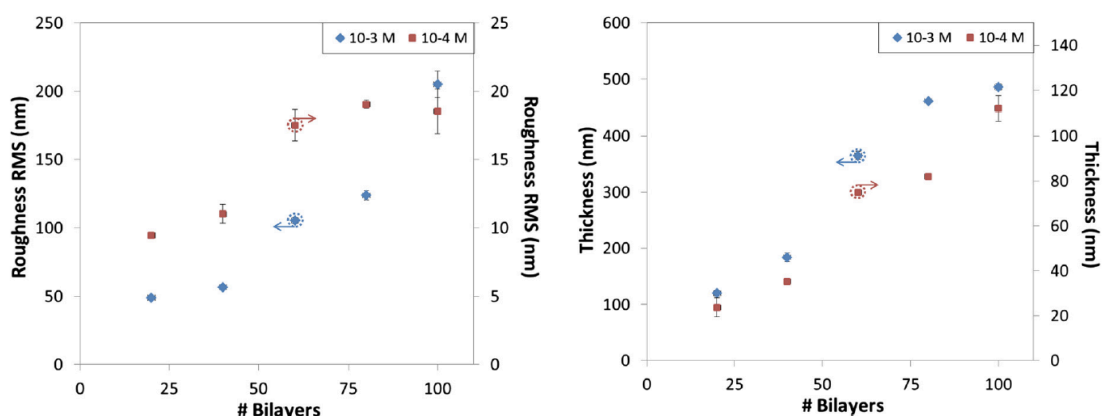


Figure 2.8. Effect of polyelectrolyte concentration³⁸ on thin film (a) roughness (b) thickness

Another dominating factor in LbL mechanism is temperature. It is known that high temperatures lead to precipitation of polyelectrolytes³⁹. Hence, by controlling raise in temperature adsorption interactions are adjusted. Increase in temperature promotes adsorption of polyelectrolytes to surface. There are various explanations regarding the effect of temperature on thin film property. According to Tan et. Al.⁴⁰ due to the increase in film swelling rise in thickness is observed. Since the polyelectrolyte chains are less viscous and the multilayer film swells more, interpenetration of coating components is more likely in the presence of high temperature⁴⁰⁻⁴¹. As well as film thickness, surface roughness also increases in the presence of raising temperature for polyelectrolyte pair⁴², since the polyelectrolyte chains develop into more coiled form⁴³.

2.3. Porphyrins and Phthalocyanine as Macrocyclic Structures

Macrocyclic compounds are defined as ring containing structures, where at least 12 atoms are present⁴⁴. There are organic and synthetic categories of macrocyclic molecules for various applications, where porphyrins (Por) and phthalocyanines (Pc) are called as structurally related cyclic tetrapyrroles. Porphyrin molecules are natural dyes, where their derivatives are present in nature in the form of chlorophyll, vitamin B12 and bacteriochlorins⁴⁵. In contrast, phthalocyanines belong to synthetic group of hydrophobic macrocycle system^{44, 46}.

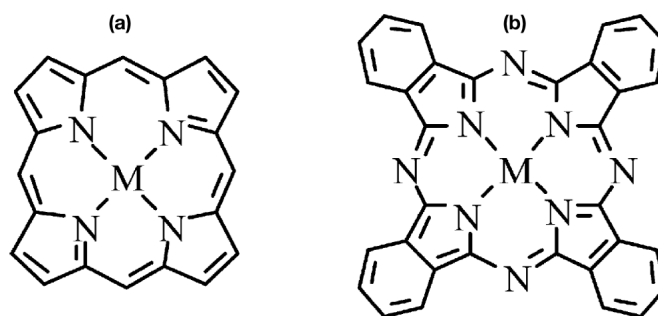


Figure 2.9. General chemical structure of (a) porphyrin (b) phthalocyanine compound⁴⁵

General chemical structures of metallated porphyrin and phthalocyanine are given in [Figure 2.9](#). Central metalation is represented by M and various metal ions may be positioned. Their electron transfer capabilities provide advantageous properties.

2.3.1. Spectroscopic Properties of Porphyrins and Phthalocyanines

Phthalocyanines show unique features by their high electrical, optical and thermal stability⁴⁷⁻⁴⁸. Because of the electron delocalization of 18π electrons, phthalocyanines gain numerous properties which make them great industrial candidates for various applications⁴⁶. Intense blue-green color is a chromatic outcome of its conjugated structure⁴⁶. Although general structures of porphyrins and phthalocyanines exhibit hydrophobic character, introduction of hydrophilic functional groups provide the desired water-soluble form. By the help of hydrophilicity, researchers acquire advantage especially in medical and biological fields⁴⁶. Due to both porphyrin's and phthalocyanine's highly conjugated structure, strong absorption appears in the visible range. The transition from n to π^* (non-bonding to anti-bonding) is attributed to the so-called Q-band (480-750nm), which is known as the highest absorption band in visible wavelength range for metallated phthalocyanines^{47, 49}. The weaker band around 380-500nm is labeled as the B-band or Soret band^{45, 47}. It is attributed to the transition from ground state to the second excited state ($S_0 \rightarrow S_1$) for porphyrins. For dissolved porphyrins/ phthalocyanines these characteristic peaks may shift or alter in intensity because of charge transfer⁴⁷.

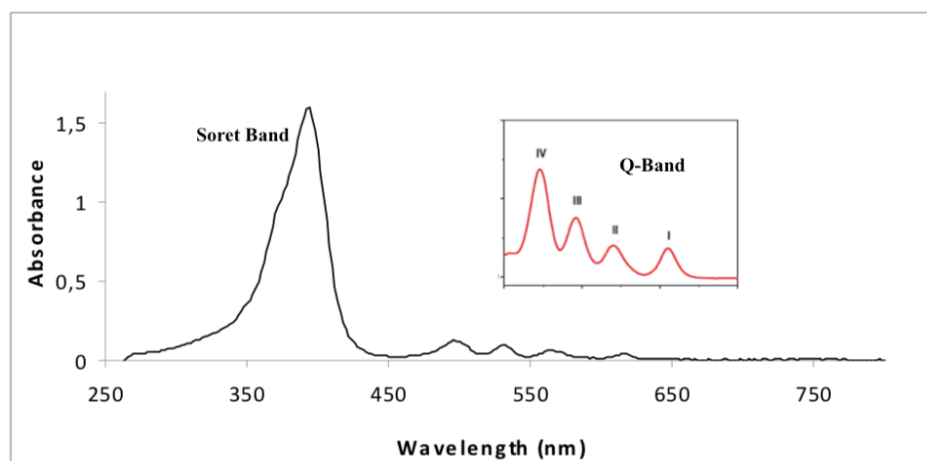


Figure 2.10. Characteristic Soret and Q bands of porphyrin molecule between 380-500 nm and 580-720 nm respectively

Unsubstituted porphyrin-based compounds are highly soluble in organic solvents such as DMSO (dimethyl sulfoxide), DCM (dichloromethane) or THF (tetrahydrofuran)⁴⁷. Addition of sulfonic acid, carboxylic acid or phosphorous-based functional groups transform the hydrophobic character of the macrocyclic compound to a water-soluble anionic structure⁴⁶. In contrast, in order to obtain a cationic hydrophilic phthalocyanine quaternization needs to be applied⁴⁶. Ammonium salts with a central nitrogen atom are great candidates for quaternary compounds.

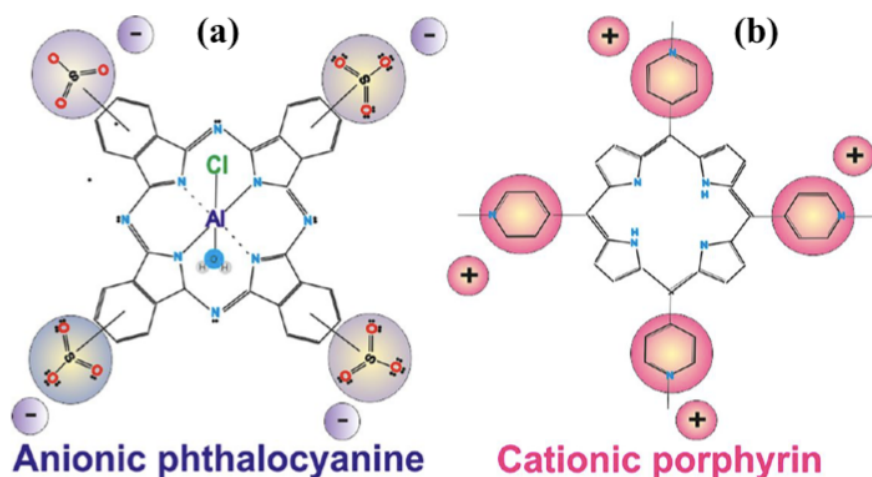


Figure 2.11. Examples for (a) anionic phthalocyanine and (b) cationic porphyrin molecules⁵⁰

2.3.2. Applications of Porphyrin/ Phthalocyanine Molecules by LbL Assembly

As well as porphyrins, also phthalocyanines are studied and applied in numerous different fields in the literature and industry. Controlled and uniform architecture obtained by LbL deposition gain interest of many researchers. Organic solar cells⁵¹⁻⁵², photodynamic therapy⁵³⁻⁵⁴, photovoltaic devices⁵⁵, biosensors⁵⁶ are only some of the application areas of macrocyclic tetrapyrroles.

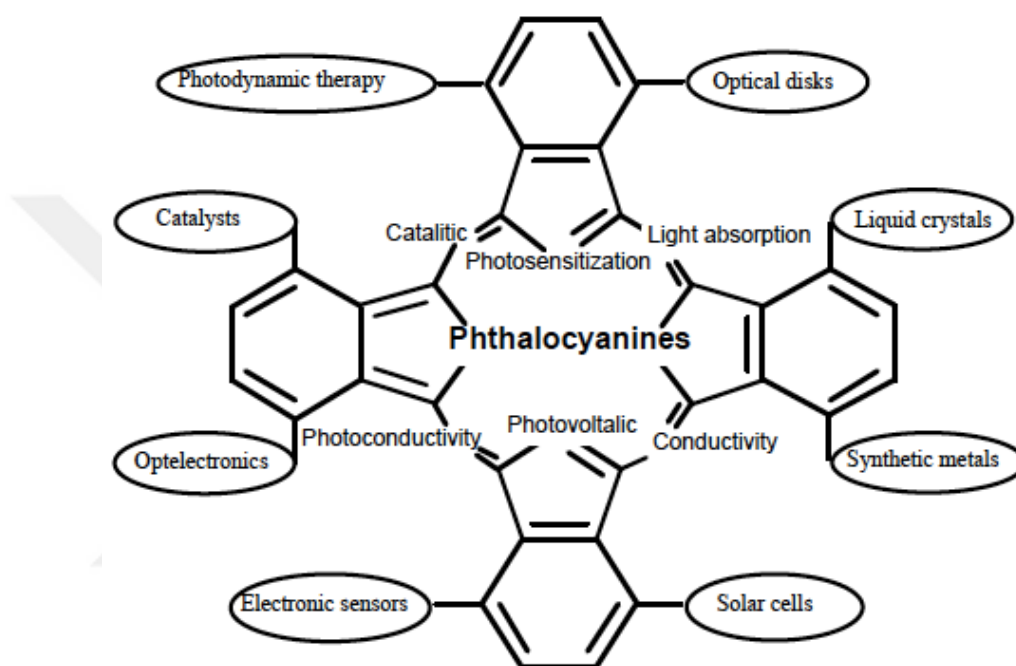


Figure 2.12. Functional application fields of phthalocyanines in literature⁴⁹

Multilayer thin films of porphyrin or phthalocyanine molecules are fabricated by layer-by-layer self-assembly since 1990s. According to the study of Crespilho et. al.⁵⁷ 20bilayer films of chitosan paired with tetra sulfonated phthalocyanine FeTsPc (iron phthalocyanine tetrasulfonic acid tetrasodium salt) and NiTsPc (Nickel-II-phthalocyanine tetrasulfonic acid tetrasodium salt) on ITO glass are coated by dip-LbL method. Formed multilayer films have exhibited high electrochemical stability, which are analyzed by cyclic voltammetry measurements. Because of the ionic interactions between amine groups and sulfonic groups, charge transport mechanism is explained by electron hopping in the study⁵⁷.

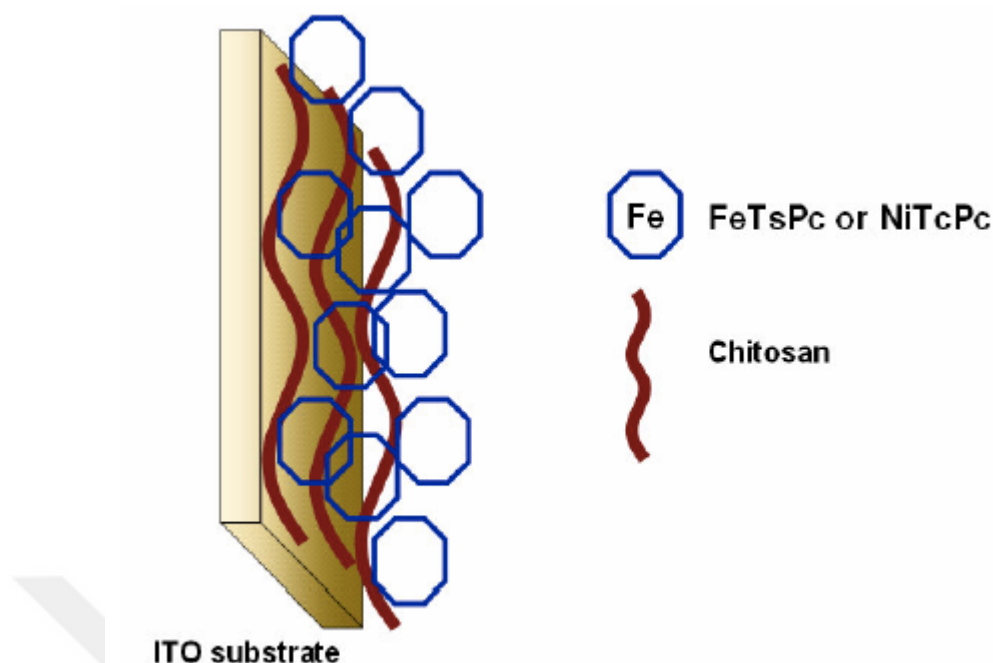


Figure 2.13. Studied chitosan/FeTsPc multilayer film structure by Crespilho et.al.⁵⁷

Similarly, multilayer films by porphyrin/ porphyrin, porphyrin/phthalocyanine and phthalocyanine/porphyrin are dip-coated by LbL technique in 1998⁵⁸. Alternated bilayer films are monitored by UV-Vis and quartz-crystal microbalance measurements. Uniform deposition in this study has proven the electrostatic interaction by absorption spectra taken after each deposition layer⁵⁸. Prepared film structures are suggested for potential photoelectric applications.

Capacity of metallated phthalocyanines in solar cell applications is widely known in literature. One of the studies in fields in 2009 by Bente et.al. revealed that multilayer thin films of poly (diallyl dimethyl ammonium chloride)/ tetra sulfonated copper phthalocyanine-CuPcTS exhibit light-harvesting and hole transporting material features⁵⁹. In addition to thin film properties, molecular orientation during deposition is investigated as well. The functionality of the LbL coating is governed by film thickness in the order of nanometers⁵⁹.

2.4. Polymeric Nanoparticles

In addition to biologists, materials scientists are also interested in the delivery mechanism of biologically active molecules or drugs to a targeted tissue. Various types of molecules such as hydrophilic/ hydrophobic drugs, genes or imaging molecules have been examined to be carried by polymeric micelles⁶⁰⁻⁶¹, liposomes⁶²⁻⁶³ or polymeric nanoparticles^{53, 64} in the literature. For high efficiency in delivery, water solubility and stability of the nanocarrier agent play a significant role. Depending on the properties of active molecule, transfer agent is selected. Either physical entrapment like hydrophobic or electrostatic interactions takes place or the drug molecule can be chemically attached to the carrier by conjugation reactions⁶⁵.

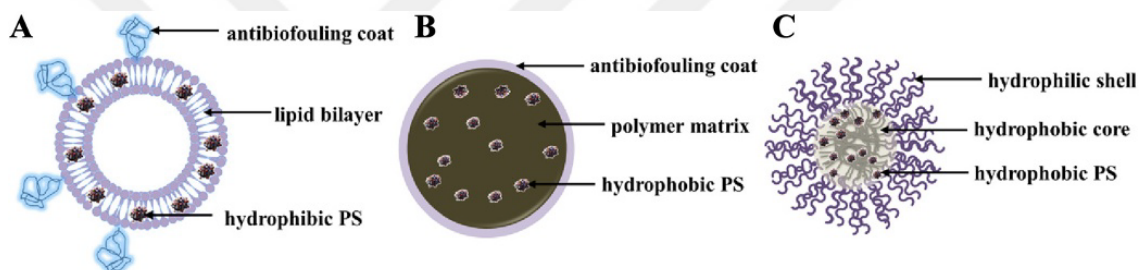


Figure 2.14. Encapsulation of active molecule by (a) liposome (b) polymeric nanoparticle and (c) micelle structure⁶⁵

In this thesis, beside hydrophilic phthalocyanine and porphyrin molecules hydrophobic zinc(II)phthalocyanine is also studied for layer-by-layer thin film formation. However, due to the water-insoluble feature of ZnPc it is quite challenging to prepare multilayer thin films of ZnPc by layer-by-layer self-assembly. Therefore, there is a need for a transfer agent. Physical entrapment is the loading mechanism of hydrophobic zinc (II)phthalocyanine into PLGA (poly D, L-lactide-co-glycolide) in this study. Due to the lack of functional groups physical entrapment is more favored for hydrophobic structures compared to chemical conjugation⁶⁶. In general, there are four main physical entrapment methods in the literature⁶⁶⁻⁶⁸, which are dialysis, oil-in-water emulsion, direct dissolution and solvent evaporation. Dialysis is one of the most functional techniques for the encapsulation of active molecule in the literature since it tolerates high-boiling point solvents like DMSO, which is replaced by water by the help of dialysis mechanism⁶⁹⁻⁷⁰.

However, certain disadvantages accompany thermodynamically stable structures. During dialysis step already loaded drug might be released from surrounding shell which decreases the loading efficiency. In addition to that, it is a time-consuming procedure compared to other physical encapsulation mechanisms. On the other hand, direct dissolution method does not require any organic solvent⁷¹. As the name implies both the drug molecule and the polymer are simultaneously dissolved in an aqueous phase under continuous stirring indicating the lack of toxic effect of an organic solvent⁷¹⁻⁷². Compared to dialysis, it provides faster release response at in vivo experiments⁷². Solvent evaporation method is one of the most common technique among physical encapsulation mechanisms. Active molecule and the polymer are dissolved in an organic solvent which has a low boiling point. Following that while the organic solvent is evaporated under vacuum, dehydration occurs for the remaining particles⁷³. For further characterization synthesized particles are collected by centrifugation⁷⁴. In solvent evaporation method solvent is the limiting factor and it does not ensure stable, spherical particle form. In this thesis, oil-in-water emulsion-solvent evaporation technique is applied for the formation of hydrophobic ZnPc loaded polymer nanospheres (Chapter 4.3). Both the drug or so-called active molecule and the encapsulating polymer are dissolved in an organic solvent. By the help of a high-energy shearing source such as sonicator or homogenizer organic phase is added dropwise to the aqueous phase which contains a surfactant or stabilizer molecule in order to form an emulsion. The obtain form is described as oil-in-water emulsion. Lastly, organic solvent is removed from the system either by continuous magnetic stirring or under reduced pressure. Molecular weight of the stabilizer molecule, type of the solvent, volume ratio of organic to aqueous phase and the sonication time are all influencing the forming micro/ nanoparticle. For further analysis, particles are centrifuged and dried.

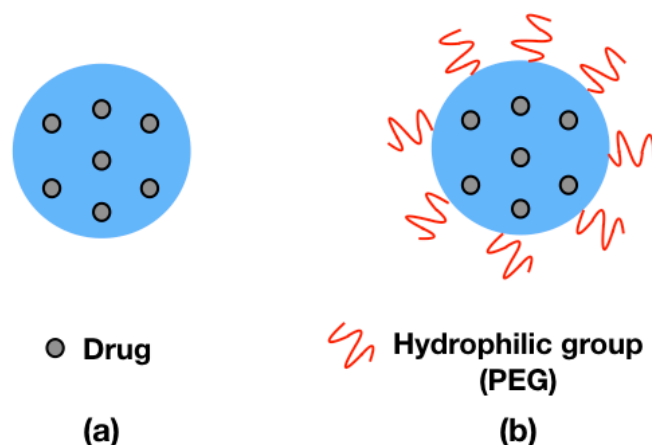


Figure 2.15. Schematic representation for encapsulation by (a) poly (D, L-lactide-co-glycolide), PLGA and (b) polyethylene glycole attached poly (D, L,lactide-co-glycolide), PEGylated PLGA⁶⁷

As the encapsulating polymer PLGA is selected due to its biocompatible^{67, 74}, biodegradable^{67, 74}, low toxicity⁶⁷ and high entrapment efficiencies⁷⁴. It is also an approved copolymer by Food and Drug Administration (FDA)⁶⁷ for drug delivery applications. There are several examples in the literature for PLGA as a polymeric carrier^{53, 65, 67, 74}. Functionalization by any hydrophilic group or polymer water-solubility of PLGA is modified as well. PEG (polyethylene glycol) is one of the frequently attached polymeric structure to PLGA to form a hydrophilic tail, which increases efficiency for drug delivery applications.

In literature, it is well-known that the driving forces for physical entrapment methods are usually electrostatic or hydrophobic interactions^{65, 75} between active molecule and the encapsulating polymer. While it is challenging for hydrophilic core molecules to maintain stability in aqueous solutions, hydrophobic drugs gain advantage by preserving their spherical structures.

Parameters influencing the size of formed polymeric nanoparticles are not only significant for biomedical, drug delivery applications but also for LbL thin film coatings. In order to have a homogeneous and uniform thin film coating material needs to be stable in aqueous medium, which requires nanometer-scaled particles. Therefore, there are certain factors that need to be considered for nano-sized particle formation rather than microparticles. Molecular weight of the polymer molecule plays a critical role on final particle size. Konan et. al represented that as the molecular weight of 50:50 PLGA

increases from 12kDa to 48kDa mean particle size is raised by 52 nanometers⁷⁶. Similarly, as the composition of D, L-lactide component of PLGA increases from 50:50 to 75:25 for 12 kDa molecular weight, particle enlarges from 102 nm to 132 nm. Like molecular weight, increase in PLGA concentration enhances the mean diameter as well⁷⁵. Sonication time is another key parameter affecting mean particle size. Prolonged sonication time decreases the overall size, since input of higher energy leads to globule breakdown⁷⁷. However, after a certain period a plateau region is obtained indicating that sufficient energy is given to split droplets⁷⁸. Stabilizing agent in the aqueous phase can also alter the final particle size. There are numerous anionic, cationic and non-ionic stabilizer types in the literature used in the emulsion-solvent evaporation technique. In this study, polyvinyl alcohol (PVA) is the only stabilizing agent added to the aqueous phase. It is reported in the literature that once the concentration of PVA in aq. phase increases nanoparticle size is expected to decrease^{75, 78}. Higher PVA amount in the organic phase-aqueous phase interface decreases the interfacial tension leading to higher net shear stress at constant energy density⁷⁸. Hence smaller nanoparticle size is acquired when PVA concentration is raised to 5% w/v from 0.5% w/v. Finally, the method to remove organic solvent is examined by Song et al as well. Removing the organic solvent by magnetic stirring leads to higher particle size formation compared to removal by rotary evaporator, which avoids aggregation⁷⁸.

3. Experimental Work

3.1. Materials

Anionic polyelectrolyte poly(sodium 4-styrene sulfonate) (SPS, $M_w = 70.000$), cationic polyelectrolytes poly(allylamine hydrochloride) (PAH, $M_w = 15.000-58.000$), branched polyethyleneimine (bPEI, $M_w = 25.000$) and poly(diallyl dimethyl ammonium chloride) (PDAC, $M_w = 100.000-200.000$) are purchased from Sigma-Aldrich. All polyelectrolyte solutions are prepared in 10 mM concentration depending on the molecular weight of their repeating unit.

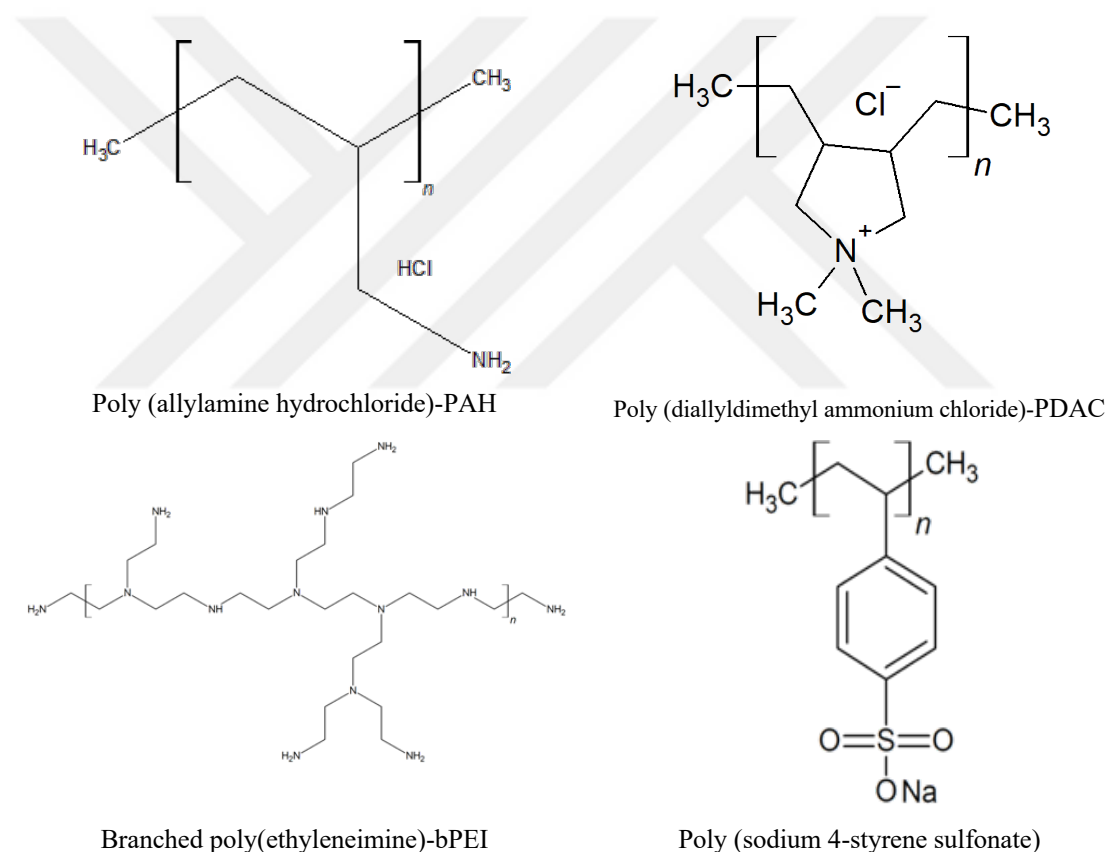


Figure 3.1. Chemical structures of studied polyelectrolytes

Nickel(II)phthalocyanine-tetrasulfonic acid tetrasodium salt (NiPcTS, $M_w = 979.40$ g/mol), 5, 10, 15, 20-(tetra-4-carboxyphenyl)porphyrin (Por, $M_w = 790.77$ g/mol), Copper phthalocyanine-3,4',4'',4'''-tetrasulfonic acid tetrasodium salt (CuPcTS, $M_w = 984.25$ g/mol), Zinc(II) phthalocyanine tetrasulfonic acid (ZnPcTS, $M_w = 898.17$ g/mol) and

Zinc (II) phthalocyanine (ZnPc, Mw= 577.91 g/mol) are studied porphyrin-based structures. Deionized water (>18MΩcm, Millipore Milli-Q) is the utilized solvent for all aqueous solutions and rinsing bath during LbL coating process. Poly (D, L-lactide-co-glycolide) (PLGA 50:50, Mn=18.000-32.000), poly (vinyl alcohol) (PVA, Mw=13.000-23.000, 98% hydrolyzed) are used for nanoparticle formation. Dichloromethane (DCM), ethanol, pyridine (all analytical grade) are purchased from Sigma-Aldrich and used for nanosphere formation and characterization.

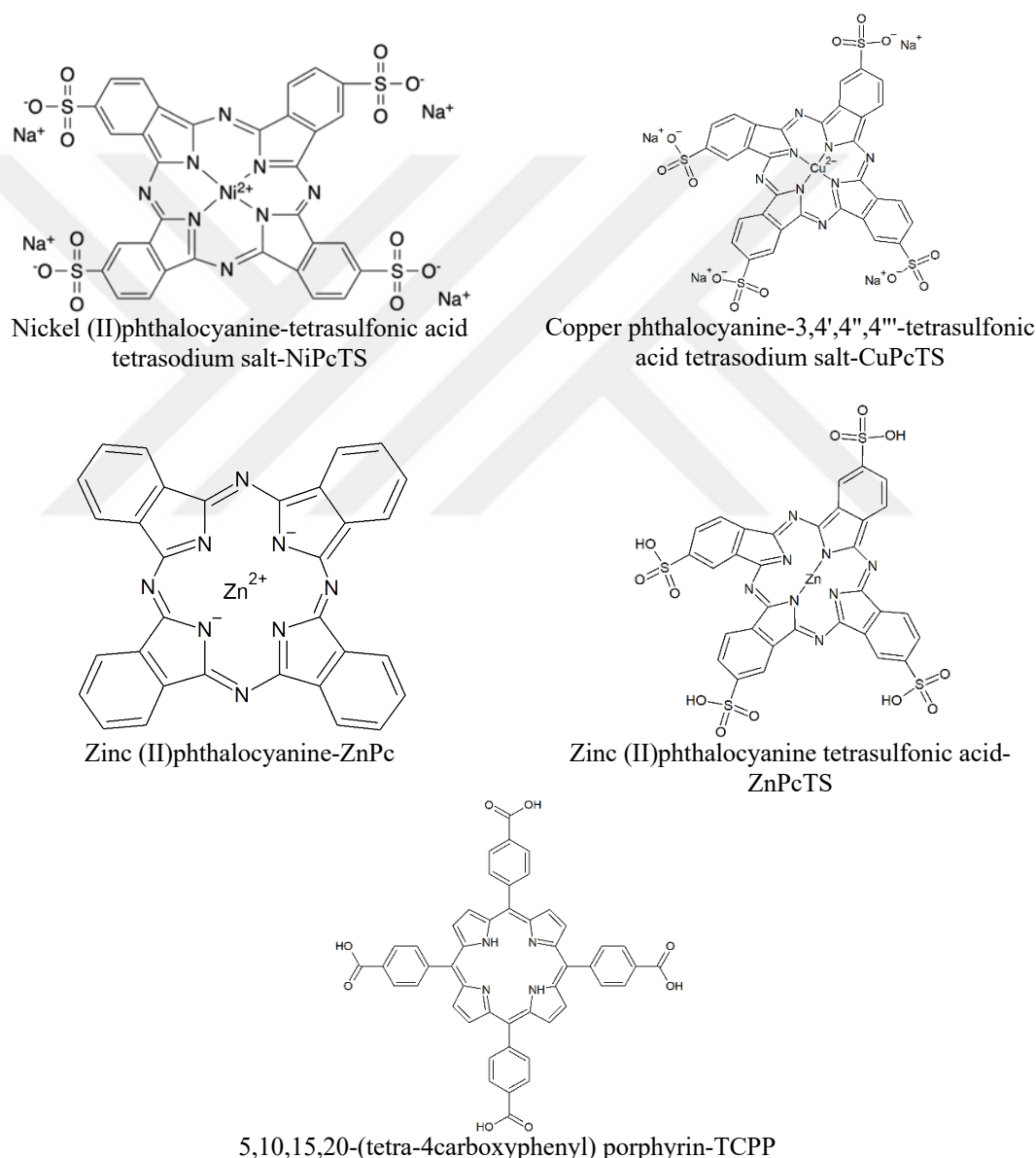


Figure 3.2. Chemical structures of studied phthalocyanine and porphyrin molecules

3.2. Experimental Methods

3.2.1. Substrate Preparation

Glass slides (75x25x1mm), Si-wafers (50x15mm) and 304 stainless steel (75x25x1mm) are studied substrates for thickness, light absorbance and electrochemical measurements by profilometer, ellipsometry, UV-Vis and potentiostat respectively. Metal substrates are cleaned by grinder with grit sizes of 400 and 600 respectively and then rinsed with acetone and water. Besides glass plates, Si-wafers and metal substrates are ultra-sonicated with Micro-90 concentrated cleaning solution-purchased from Sigma-Aldrich- and distilled water for 20 min each respectively. Once all slides are rinsed with ethanol/ water respectively, substrate surfaces are dried with nitrogen gun before any plasma cleaning for 3-4 min by Harrick Plasma Cleaner.

3.2.2. Layer-by-Layer Self-Assembly

Concentration of all polyelectrolyte solutions (PAH, PDAC, bPEI, SPS) are set to 10mM depending on the molecular weight of their repeating unit. Depending on the purpose and targeted application phthalocyanine and porphyrin molecules are studied with 0.1, 0.25 and 1mM concentrations. Strong polyelectrolytes do not require any modification in pH, therefore PDAC is used with its intrinsic pH value and pH of SPS is 4.00. Weak polyelectrolytes are adjusted to pH 4.00. Depending on their solubility in aqueous medium, pH of the porphyrin is set to 10.7. CuPcTS, NiPcTS and ZnPcTS are studied with their intrinsic pH values. Besides, influence of NiPcTS pH on LbL thin film properties is investigated in [Chapter 4](#). Deionized water is the solvent molecule for all studied polyelectrolyte, phthalocyanine and porphyrin structures.

Once the necessary substrate (glass, ITO coated glass, si-wafer or stainless steel) is prepared, it is dip-spun by our special designed LbL automation system ([Figure 3.3](#)), where 100rpm is the spin rate, 10 min is dipping duration for coating material and 2/1/1 min dipping durations for wash baths respectively. Following each coating procedure thin films are dried by nitrogen gun for further characterization.

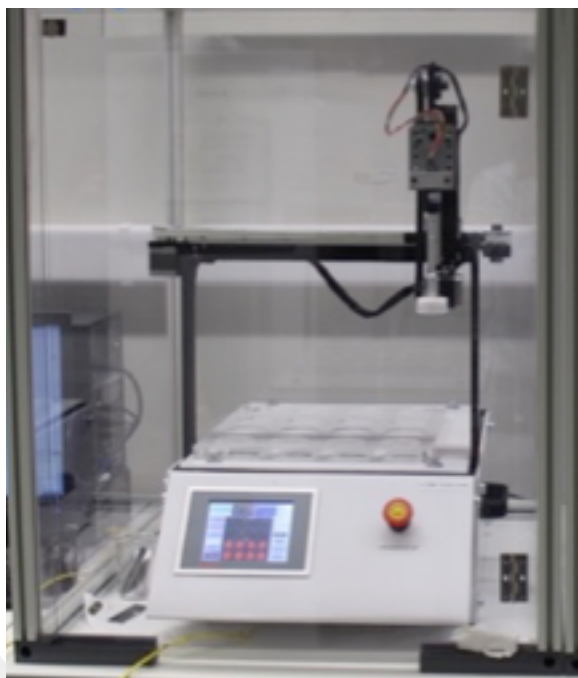


Figure 3.3. Automated dip-spin LbL device

3.3. Nanosphere Preparation Mechanism

Among several physical entrapment methods oil-in water (o/w) emulsion-solvent evaporation technique is applied for the encapsulation of hydrophobic zinc phthalocyanine by poly (D,L-lactide-co-glycolide), PLGA in Chapter 6. Same experimental steps in literature are followed. An organic phase and an aqueous phase are required. In the organic phase zinc (II) phthalocyanine as the active molecule and PLGA as the encapsulating polymer are dissolved in dichloromethane (DCM) under ultrasonication. In a separate beaker, polyvinyl alcohol (PVA) as the stabilizing agent is dissolved in distilled water. Organic phase is added to aqueous phase dropwise while mixing with IKA Ultra-turrax T18 homogenizer at 20400 rpm. When all organic phase is added prepared emulsion is probe sonicated at 50 W output for 5 min to decrease particle size from micron to nano. Dichloromethane has a low boiling point at 39°C, therefore it is removed from the emulsion by magnetic stirring under room temperature for 4 hours.

In order to remove the stabilizing agent from system, centrifugation for 15 minutes is applied at 10.000 rpm and resuspended in water. Centrifugation is repeated for 3 times.

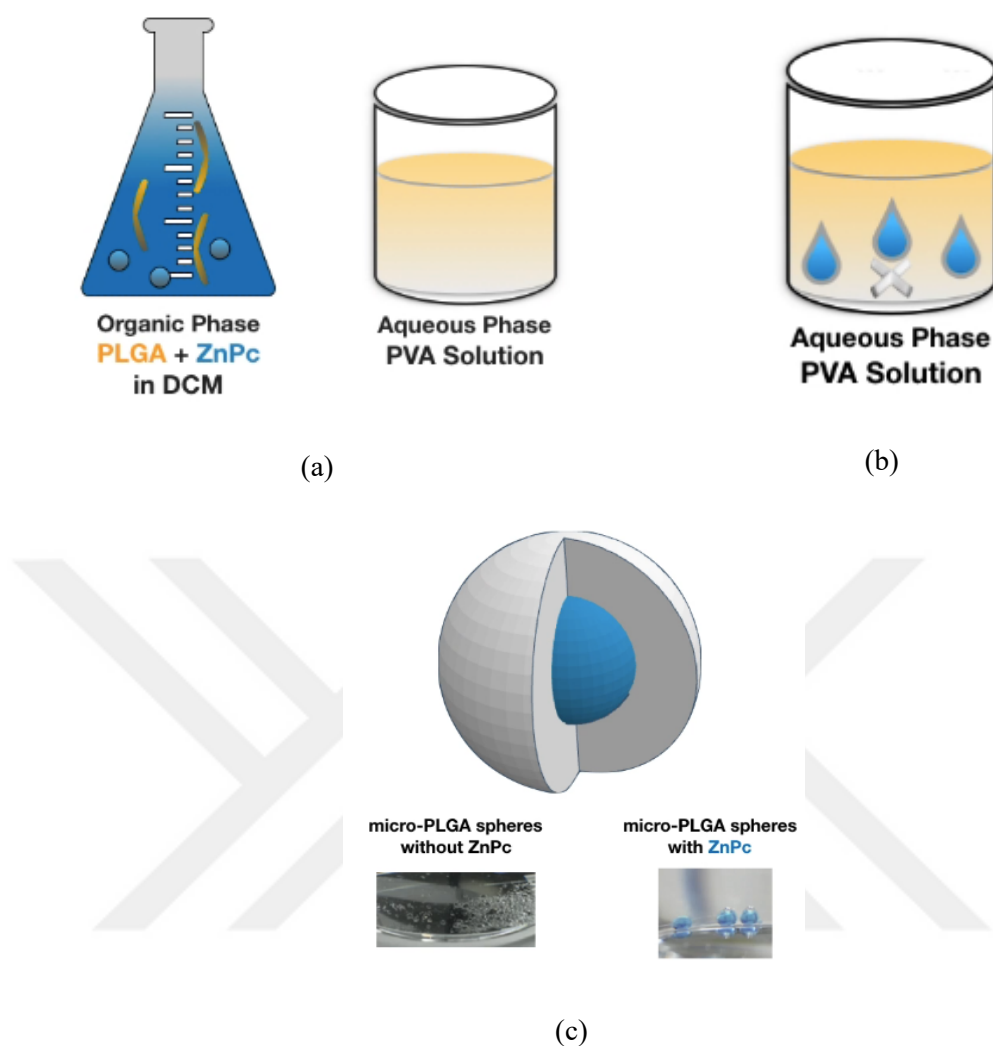


Figure 3.4. Step-by-step nanosphere formation (a) content of the organic and aqueous phases, (b) microemulsion formation while adding organic phase to aqueous phase, (c) final capsule structures

3.4. Characterization Methods

3.4.1. Dynamic Light Scattering (DLS)

Zetasizer Nano ZS, Malvern Instruments Ltd. is used for dynamic light scattering (DLS) measurements. Hydrodynamic size and zeta potential measurements are performed for the encapsulated nanospheres and for the pH studies of phthalocyanine molecules. 0.3 g/L is the prepared concentration for phthalocyanine encapsulated nanosphere formulation, which is presented in [Chapter 6](#). For the zeta potential analysis of phthalocyanines 10^{-3} M aqueous solutions are prepared. pH of all solutions is adjusted

by HCl and NaOH solutions. All measurements are performed at 25°C by 5 times where each one run 11 cycles. Disposable low volume polystyrene cuvettes and disposable folded zeta cuvettes are used for size and zeta potential measurements respectively. Mean particle size and number percentage are considered. Surface charge of particles are examined by zeta potential analysis.

3.4.2. Electrochemical Measurements

PARSTAT MC potentiostat is used for all electrochemical measurements. Cyclic voltammetry, Tafel and electrochemical impedance spectroscopy analysis are performed in an electrolyte of diluted Harrison's solution (0.35wt% $(\text{NH}_4)_2\text{SO}_4$ + 0.05wt% NaCl), which almost simulates the atmospheric conditions for aerospace vehicles. All measurements are performed under room temperature.

3.4.2.1. Tafel

Tafel measurements are performed to investigate anodic, cathodic responses and corrosion behavior of LbL coated multilayer films. Stainless steel is the coated substrate surface. Gamry Corrosion Paintcell is used as experimental setup (Figure 3.5). Ag/AgCl in saturated NaCl solution and graphite rod are the reference and counter electrodes for measurement system respectively. 1cm^2 is the working electrode area. Initial and final potential values are adjusted as -0.25 V and +0.25 V with respect to open circuit potential respectively. Scan rate is 1 mV/s for each measurement. Corrosion rate, corrosion potential and corrosion current density values are computed according to potential vs current density diagrams.

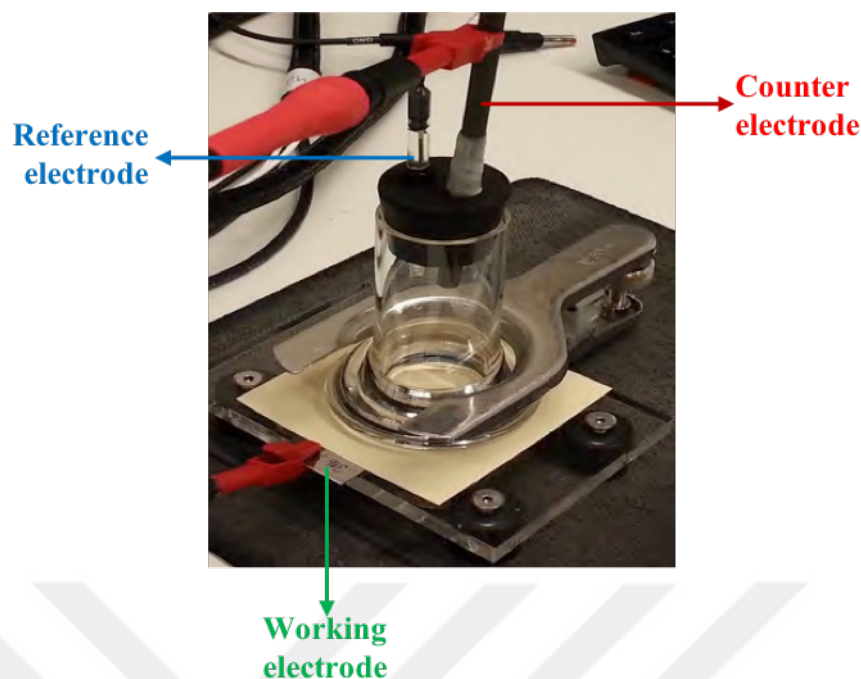


Figure 3.5. Gamry Corrosion Paintcell setup

3.4.2.2. Cyclic Voltammetry (CV)

Cyclic voltammetry (CV) measurements are performed to examine electroactivity of phthalocyanine molecule among fabricated multilayer LbL films. Thin films on ITO (indium-tin oxide) coated glass substrates are investigated. Following substrate pre-treatment, linear poly (ethyleneimine) is dip coated as an initial sticking layer. On top of that standard layer-by-layer film coating procedure is applied. Instead of Paintcell, standard three-electrode setup is preferred. Reference and counter electrodes are Ag/AgCl in saturated NaCl and platinum plate respectively. Working electrode area is 0.4cm^2 . Scanning is performed between -0.3V to 1V with 20 mV/s scan rate. Diluted Harrison's solution is the electrolyte in system. Oxidation and reduction behavior of multilayer coating is investigated according to current vs potential response.

3.4.2.3. Impedance Spectroscopy (EIS)

Electrochemical impedance spectroscopy measurement is performed to determine capacitance and resistance tendency in multilayer films. The same experimental setup is used with Tafel measurement. Frequency range is between $1\text{-}10^5\text{ Hz}$. In order to stabilize open circuit potential each stainless-steel substrate is immersed in the Harrison's solution

for 1 hour. Amplitude is adjusted as 10 mV. Nyquist, Bode and phase angle diagrams are analyzed for further interpretation.

3.4.3. Scanning Electron Microscopy (SEM)

As well as surface characterization of fabricated thin films, nanosphere formation is also monitored by Zeiss LEO Supra 35VP field emission scanning electron microscopy. Nanospheres in powder form, LbL films coated on glass and Si-wafer substrates are analyzed. Nanosphere containing powder samples are directly stuck on to two-sided carbon tape, which is placed on SEM stub, for electron conductivity. Similarly, prepared thin films on any substrate is cut by diamond cutter to stick on SEM stub. Prior to observation each sample is coated with gold/palladium target by Desk V HP, Denton Vacuum sputtering machine for 2-3 minutes to get electron-conductive surface. Finally, SEM stubs are placed to SEM sample stage. Secondary electron detector is used to monitor samples powered 3-5 keV within 8-10 mm working distance. Surface morphology and size distribution of nanospheres are explored according to the obtained secondary electron images.

3.4.4. Surface Profiler (Profilometry)

In order to comment on multilayer thin film thickness KLA Tencor P6 surface profiler is used. At least 7 different measurement points are picked on a sample for mean thickness value. Thin films on glass substrates are scratched prior to any measurement. According to the difference in height thickness data is collected.

3.4.5. Spectroscopic Ellipsometry

As an alternative to surface profiler measurements, thickness of the prepared thin films is examined by J. Woolam M2000 spectroscopic ellipsometer. According to the working principle change in polarization state of incident light beam is measured depending on the reflection from specimen. Detector collects and measures the amount of polarized light after reflection from specimen in terms of amplitude and phase shift. Studied

wavelength range is between 315-718 nm. Different from surface profiler, Si-wafer is the coated substrate in this thesis. In order to compute an average value at least seven distinct points are evaluated. Cauchy and b-spline models are employed to fit thickness data.

3.4.6. Quartz-Crystal Microbalance (QCM)

Adsorption mechanism of each deposited layer is monitored by Q-Sense E1 quartz-crystal microbalance (QCM) detector. By the help of piezoelectric properties of quartz sensor, frequency is changing due to adsorbed material's mass. The relationship between change in frequency of quartz-crystal and the change in mass of adsorbed specie is calculated by the Sauerbrey's equation. In addition, change in dissipation is also determined according to the viscoelastic properties of coating material.

$$\Delta m = -C * \frac{1}{n} * \Delta f \quad (3.1)$$

LbL assembly is applied on 5 MHz, 14mm AT-cut sensor with gold electrodes to quantify adsorbed material amount coated on the surface. Flow rate for each coating material is 0.1 ml/min. Before any measurement, quartz crystal is treated with UV/ozone generator for 10 min. Following that crystal is dipped in a preheated (approximately 75°C) cleaning bath mixture, which is made of 5:1:1 distilled water, ammonia (%25) and hydrogen peroxide (%30). Quartz is treated in the hot bath for 5 min and rinsed by milliQ water. Nitrogen gas is used to dry sensor surface before UV/ozone treatment for another 10 minutes before running an experiment. Similar to layer-by-layer coating method on any substrate, quartz-crystal surface is coated with 10 mM LPei polyelectrolyte as an initial layer for 10 minutes. Following that crystal surface is rinsed with milliQ water for 4 minutes. LbL thin film deposition procedure is applied subsequently. While introducing oppositely charged coating materials in a LbL deposition sequence, frequency and dissipation changes are simultanelously collected to estimate the change in film thickness. Measurement and LbL deposition are executed under room temperature.

3.4.7. Ultraviolet-visible Spectroscopy (UV-Vis)

Due to the blue-green color of studied phthalocyanine structures film growth is examined by change/ increase in absorbance in the visible wavelength range. As well as Cary 5000

UV-VIS-NIR, Shimadzu UV-3150 spectroscopy is also used for both solid (thin film) and aqueous solution absorbance analysis. Disposable polystyrene cuvettes are used for liquid phthalocyanine and porphyrin samples. Once the desired concentration is prepared, 2-3 milliliters are subjected to UV-vis analysis between 300-800 nm. Similarly, glass substrates which are homogeneously coated by LbL technique are exposed to absorbance measurement in the visible wavelength. MilliQ water and blank glass substrates are reference materials for aqueous and solid samples respectively. Baseline correction is applied before each absorbance assessment. Solid sample holder is replaced with the liquid sample/ cuvette holder of Cary 5000 spectroscopy for thin film analysis. Once the absorbance data is collected, film growth with respect to number of layers is plotted. Furthermore, wavelength where the maximum absorbance observed is compared with the literature values.

3.4.8. Fluorescence Emission Spectroscopy

Fluorescent properties of phthalocyanines allow detection of fluorescence emission spectrum in the visible wavelength range. Especially for encapsulation experiments efficiency of surrounding polymer-loaded phthalocyanine relationship can be monitored by fluorescence spectroscopy. For this purpose, Cary Eclipse fluorescence spectrophotometer is used. 2-3 milliliters of zinc phthalocyanine and encapsulated zinc phthalocyanine, dissolved in an organic solvent, are measured in quartz cuvette separately. Sample is excited at 640 nm and the corresponding fluorescence emission data is collected.

3.4.9. Atomic Force Microscopy (AFM)

Especially for thin films homogeneity and surface roughness are significant outcomes for a favorable coating. Surface topography of deposited nanospheres on Si-wafer and glass substrates is investigated by Bruker MultiMode8 atomic force microscopy in tapping mode and compared with the topography of standard polyelectrolyte film. Scan rate is 0.5 Hz with 256 sample lines. 1 μm x 1 μm area is scanned.

4. Investigation of pH and concentration influence on layer-by-layer self-assembly for nickel (II) phthalocyanine-tetrasulfonic acid tetrasodium salt coatings

4.1. Introduction

Phthalocyanines and porphyrins, which display analogous molecular structures because of their aromatic character, draw attention of many researchers due to their highly conjugated π -electron structures⁷⁹ with colorful appearances. These aromatic macromolecules containing a metallic-core are labeled as metallated-phthalocyanines/-porphyrins. It is well known that phthalocyanines and their derivatives exhibit significant optical⁷⁹, electrochromic⁸⁰, photodynamic⁸¹⁻⁸² features in the literature. In the presence of hydrophilic functional groups attached to porphyrins and phthalocyanines their aqueous phase allow low-cost, feasible and promising surface-based application methods. Due to their diversity in molecular structure and functionality porphyrins and phthalocyanines intrigued interest of material scientists.

Among several surface coating methods such as Langmuir-Blodgett (LB) and self-assembled monolayers (SAM), layer-by-layer (LbL) deposition technique is commonly preferred due to its water-based, simply-applicable and environmental-friendly properties⁴³. It is possible to obtain multilayer thin films where the film thickness is controlled in the order of nanometers. One of the primary requirements for a conventional LbL film is opposite electrostatic interactions while there are several factors³¹ reported in the literature that contribute to the homogeneous and uniform LbL coating morphology. Concentration^{31, 38}, pH^{13, 37} or ionic density and temperature⁴¹ of coating materials play a significant role on molecular conformation on surface; hence on multilayer film thickness and uniformity. Besides polyelectrolytes, nanoparticles, bio-macromolecules⁸³⁻⁸⁵, inorganic^{17, 86-87} and organic compounds are easily embedded into multilayer systems. Layer-by-layer coatings serve for biological sensors^{21, 88}, antireflective coatings⁸⁹ and corrosion⁹⁰⁻⁹² purposes beside other fields.

In the literature, layer-by-layer films of phthalocyanine-based compounds are studied for various utilizations such as temperature-dependent coatings⁹³, polyphenol detection⁹⁴, photo-voltaic⁵⁹ properties etc. Benten et al.⁵⁹ has reported thin-film solar cells by using

copper(II) phthalocyanine-3,4',4'',4'''-tetrasulfonic acid tetrasodium salt (CuPcTS), where the thickness of prepared films affect photovoltaic behaviour. In another study by Sergeeva et al⁹³, CuPcTS containing LbL films are tested for their high-temperature performance. Investigation of change in the pH condition of NiPcTS is essential for potential surface-based applications and it provides unique results. pH of the aqueous phthalocyanine affects thin film growth strongly while the ionic density varies. Once the ionic concentration of NiPcTS embedded into the film system is adjusted, it is possible to transfer controllable amount of phthalocyanine to surface by LbL technique.

In this study, effect of pH and concentration on nickel(II)phthalocyanine-tetrasulfonic acid tetrasodium salt (NiPcTS) is studied for multilayer thin film formation by layer-by-layer method. Film growth is examined by light absorbance and thickness measurements. Surface adsorption of NiPcTS at acidic medium is also monitored by quartz-crystal microbalance.

4.2. Materials & Methods

4.2.1. Materials

As anionic polyelectrolyte poly(sodium 4-styrene sulfonate) (SPS, Mw= 70.000) and as cationic polyelectrolyte poly(allylamine hydrochloride) (PAH, Mw= 15.000) and branched polyethyleneimine (bPEI, MW= 25.000) molecules are used. Polyelectrolyte concentrations are set to 10 mM, which are based on the molecular weight of the repeating unit. Aqueous solutions of SPS, PAH and bPEI polyelectrolytes have pH value of 4.00. Nickel(II)phthalocyanine-tetrasulfonic acid tetrasodium salt (NiPcTS, Mw= 979.40 g/mol) is used as an anionic phthalocyanine molecule. Concentration of NiPcTS is adjusted to 0.1 mM and 0.25 mM, where pH of NiPcTS varies between 2.50/ 5.50/ 7.50 and 10.50. All of the chemicals are purchased from Sigma Aldrich and their corresponding molecular structures are given at [Figure 4.1](#). Distilled water is the solvent of all materials.

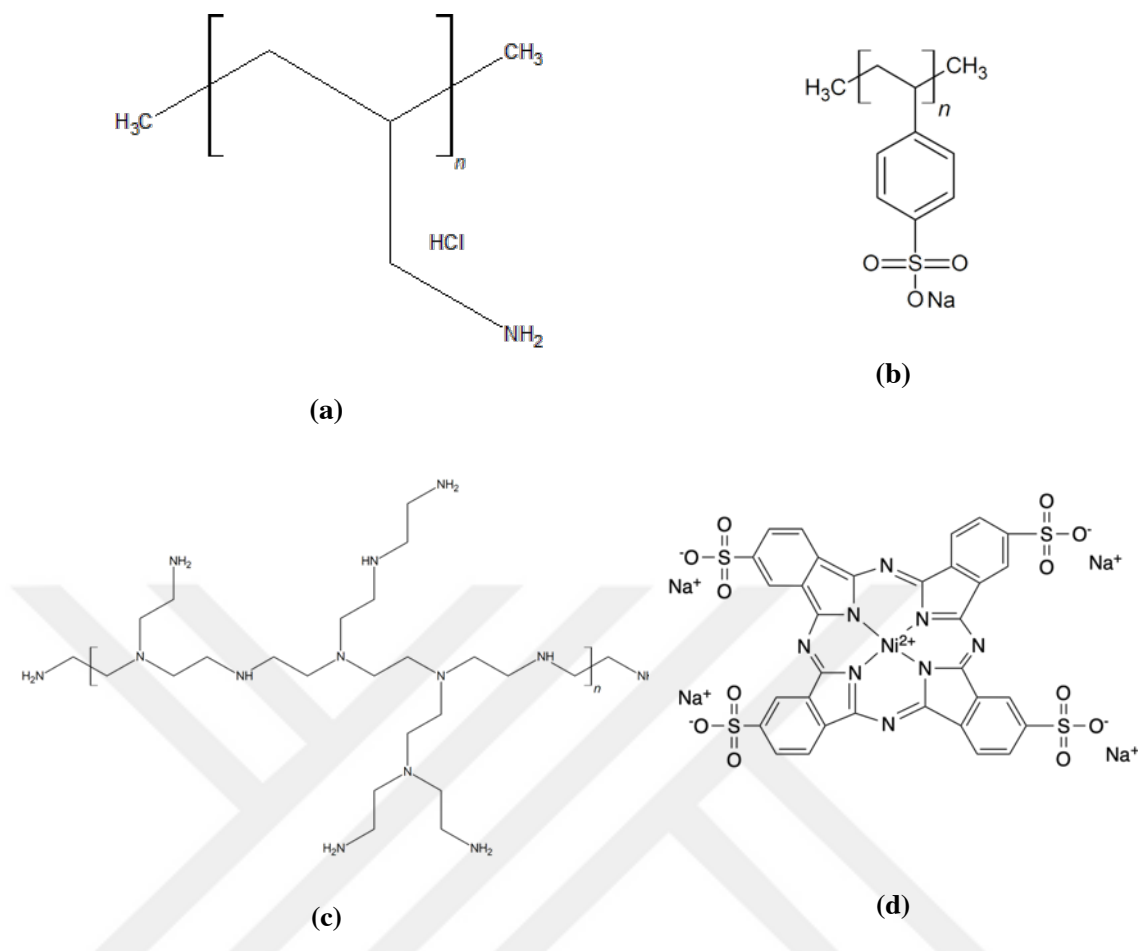


Figure 4.1. Molecular structures of studied polyelectrolytes and phthalocyanine. (a) poly(allylamine hydrochloride)-PAH (b) poly(sodium 4-styrene sulfonate)-SPS (c) branched poly(ethyleneimine)-bPEI (d) Nickel(II)phthalocyanine-tetrasulfonic acid tetrasodium salt-NiPcTS

4.2.2. Layer-by-Layer Multilayer Thin Film Formation

Multilayer thin films are obtained by layer-by-layer (LbL) deposition method. Coating procedure is initiated with the PAH/SPS polyelectrolyte pair, which is designed to be adhesive layers. Once glass slides are cleaned as described above, substrates are dip-spin coated by 10 mM aqueous PAH solution for 10 min at 100 rpm. Excess of the charged molecules on the coating is removed by dip-spinning the substrate in three different distilled water baths for 2/1/1 min respectively. Following that, anionic adhesive layer, SPS is dip-spin coated for another 10 min at 100 rpm and the substrate is rinsed again with distilled water three times. This one cycle is called as one bilayer (bL) and five bilayers of adhesion pair (PAH/SPS) is dip-spin coated on both surfaces of glass slides. On top of that the same coating procedure is repeated for desired number of layers with

(bPEI/ NiPcTS)_n pair. Aqueous solution of bPEI is distributed on precoated SPS surface for 10 min at 100 rpm. Rinsing by distilled water is applied in three different wash-baths as 2/1/1 min respectively. Following that, substrate is dip-spinned in anionic aqueous solution of NiPcTS in selected pH value for another 10 min at 100 rpm. Finally, excess of coating material is washed out by other rinse-baths as 2/1/1 min respectively. Varying the pH value of anionic NiPcTS thin film formation behavior is analyzed.

4.2.3. Characterization

Thin film growth on glass slides is investigated by Cary 5000 UV-VIS-NIR spectroscopy in the visible range. Thickness measurements of multilayer coatings are performed by KLA Tencor P6 surface profiler and at least seven different points on each film is measured. Adsorption kinetics is measured by Q-Sense E1 quartz-crystal microbalance (QCM). LbL thin film assembly is applied on 5 MHz, 14 mm AT-cut sensor with gold electrodes. Change in frequency and dissipation is recorded simultaneously with LbL coating under room temperature.

4.3. Results & Discussion

4.3.1. General LbL Film Formation

Layer-by-layer self assembly is a water-based coating mechanism, where intermolecular interactions such as electrostatic attraction, hydrogen or covalent bonding is utilized. Since NiPcTS is water-soluble in a wide range of pH, four different pH conditions are studied to investigate its effect on thin film formation. In [Figure 4.2](#) a general protocol for LbL process is demonstrated. Any substrate, where water can diffuse, is dip-spinned in positively charged polyelectrolyte solution.

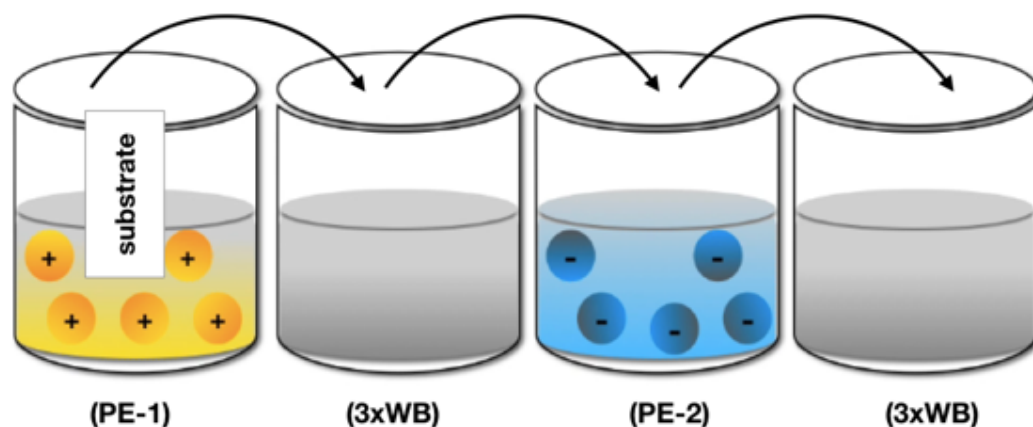


Figure 4.2. Layer-by-layer (LbL) coating mechanism, PE-1: positively charged polyelectrolyte, WB: water-bath, PE-2: negatively charged material, containing NiPcTS during film coating process.

In our case PAH and bPEI are the initial cationic aqueous solutions for adhesion and coating processes respectively. Substrate is dip-spun in (PE-1), which is PAH solution for adhesion and bPEI polyelectrolyte solution for coating process, for 10 min at 100rpm. Next, excess material on the surface of substrate is removed by dip-spinning the substrate in three different water-baths for 2/1/1 min respectively. Following that, oppositely charged material is introduced as (PE-2), which is SPS solution for adhesion and NiPcTS solution for coating process. After 10 min dip-spin at 100 rpm substrate is washed with three water-baths again. Once all these four steps are completed, one bilayer (bL) coating is accomplished. It is possible to repeat the LbL process as desired number of layers is obtained.

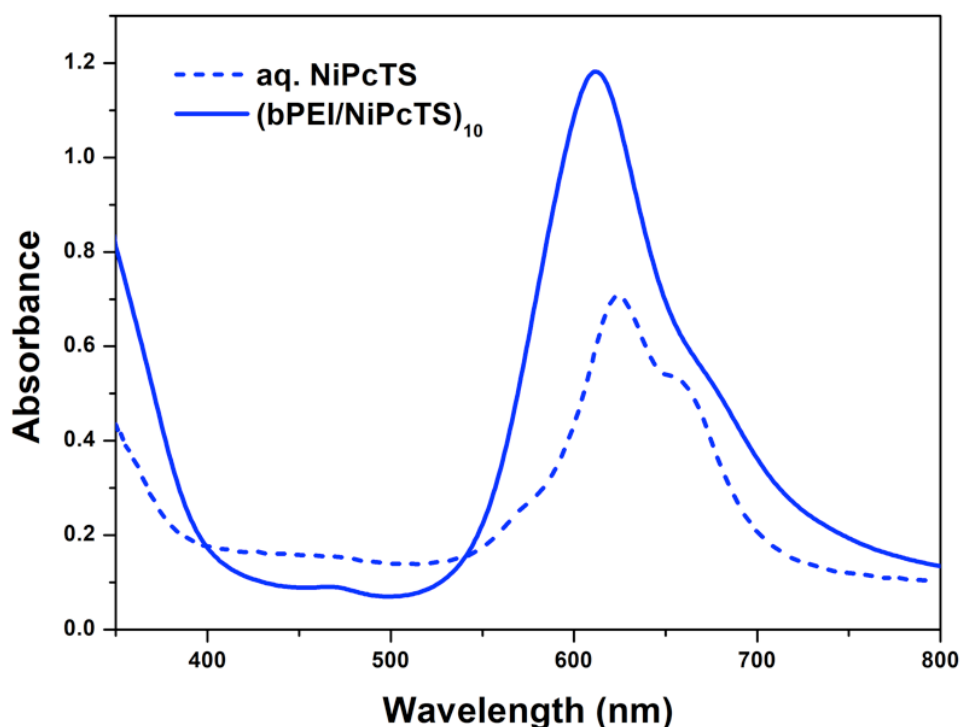


Figure 4.3. Film formation shown by absorbance spectra of liquid and solid phases, where dashed curve (---) represents aqueous NiPcTS solution and solid curve (—) represents 10 bilayers of thin film (bPEI/NiPcTS). pH value for NiPcTS is 2.5

4.3.2. Aqueous phase vs Solid Film

As a primary requirement of LbL technique, coating materials need to be water-soluble. Aqueous solutions of studied polyelectrolytes (PAH, SPS and bPEI) and NiPcTS are easily prepared due to their attached amine and sulfonate groups. A set of pH values between 2.5-10.5 enables homogeneous aqueous solutions for NiPcTS. Because of the electrostatic interactions between polyelectrolytes and phthalocyanine compound layer-by-layer self-assembly leads to thin film formation. Taking advantage of the colorful appearances of phthalocyanine, multilayer film growth is examined by UV-VIS spectroscopy in the visible wavelength range. Color changes from light blue to dark blue for NiPcTS embedded films indicate film growth as well. Absorbance spectra of both liquid and solid phases of NiPcTS are compared. According to the [Figure 4.3](#), both aqueous solution (0.01 mM) and 10 bilayers film of NiPcTS (0.25 mM) give absorbance

maximum at 614 nm represented by blue dashed and solid curves respectively. Q-band of NiPcTS is clearly displayed by an intense absorption peak at 614 nm, which arises because of the transition between π - π^* of phthalocyanine. Hence, [Figure 4.4](#) is plotted to show film growth by visible light spectroscopy precisely. As expected, as the number of layers increases from 2 bilayers to 10 bilayers, intensity of Q-band of (bPEI/NiPcTS)_n increases as well.

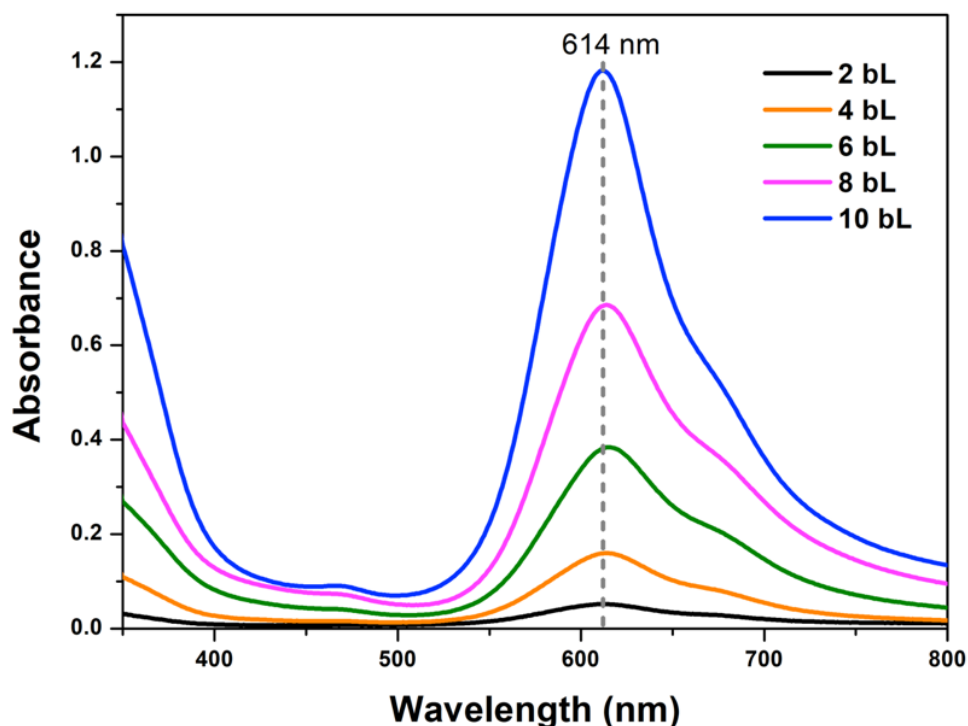


Figure 4.4. Multilayer thin film growth of (bPEI/NiPcTS)_n coating system by UV-VIS spectroscopy. Q-band maxima is obtained at 614 nm wavelength

4.3.3. Influence of concentration on LbL self-assembly

One of the critical parameters affecting thin film thickness and growth at layer-by-layer self-assembly is concentration. By changing the concentration of coating material it is possible to gain control over thin film thickness. Similar to the literature^{13, 32}, polyelectrolyte concentrations are set to 10 mM (considering the molecular weight of the repeat unit). NiPcTS has an intense character in aqueous phase. Various concentrations of phthalocyanine are studied in literature^{59, 93, 95} for LbL self-assembly. Rather than the

intensity of absorbance, where maximum absorbance appears plays a significant role for characterization by spectroscopy. Therefore, even few millimolar concentrations of phthalocyanine is sufficient for analysis. In our study, NiPcTS is prepared in two different concentration conditions, at 0.25 mM and 0.1 mM. For each case multilayer thin films of bPEI/NiPcTS pair are prepared up to 10 bilayers. Effect of concentration is measured in terms of absorbance in the visible wavelength range and film thickness by surface profiler. In [Figure 4.5](#) and [Figure 4.6](#) multilayer thin film properties containing 0.25 mM NiPcTS are demonstrated by red and 0.1 mM NiPcTS by blue points. There is a similar exponentially increasing trend in thickness and absorbance intensity for both concentration cases. 10 bilayers of (bPEI/NiPcTS) film assembled from 0.25 mM NiPcTS concentration (pH 2.5) brings 98.3 nm thickness while 0.1 mM NiPcTS gives 80.9 nm on glass surface. In addition, effect of concentration on thin film absorbance is plotted in [Figure 4.6](#). Up to 6 bilayers of (bPEI/NiPcTS), both 0.25 mM and 0.1 mM NiPcTs conditions display almost identical absorbance characteristics. However, as number of bilayers increases to 10, multilayer thin film formed by higher concentration diverges faster. As expected, higher concentration exposes higher thickness and absorbance values. Nevertheless, there is not 2.5 fold increase in thin film properties as the concentration of the anionic layer NiPcTS is raised by 2.5 fold.

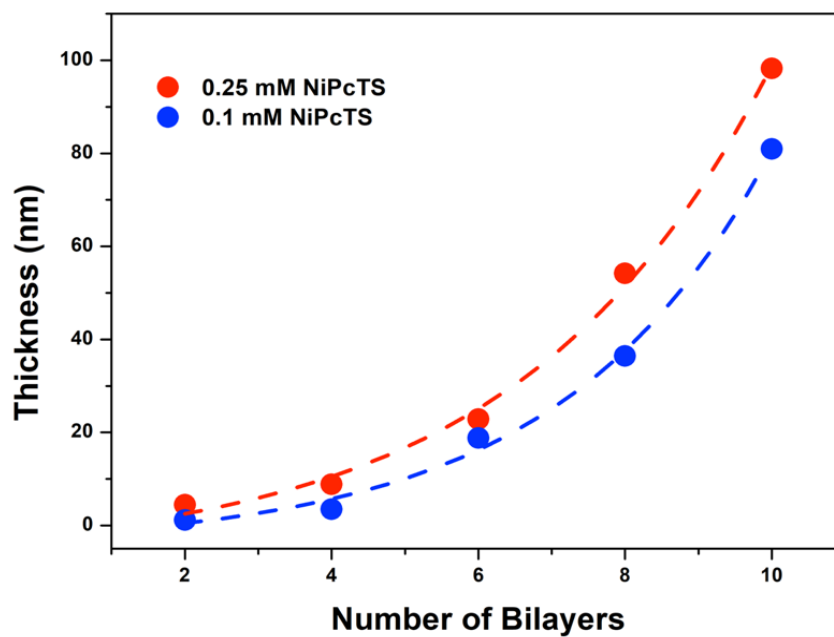


Figure 4.5. Thin film growth demonstrated by change in thickness for two different concentration conditions of NiPcTS (pH=2.5 in both cases)

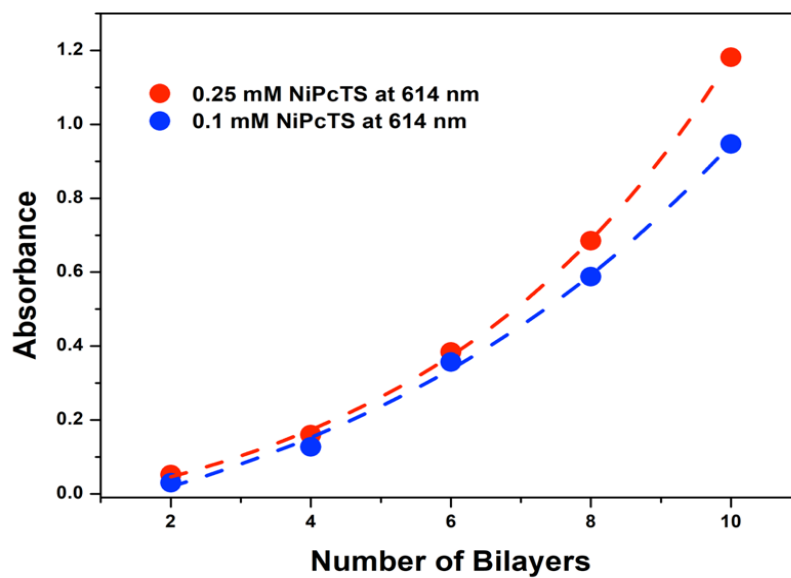


Figure 4.6. Thin film growth demonstrated by change in absorbance for two different concentration conditions of NiPcTS (pH=2.5 and $\lambda=614$ nm in both cases)

The reason behind enlightens the exponential growth as well. There might be two explanations for the exponential behavior. First of all, since one of the coating materials, polyelectrolyte bPEI or the metallated phthalocyanine swell and diffuse into the previously coated layer, there is not any visible distinctive layer distribution. Each added layer interpenetrates into underneath layer until electrostatic charge saturation is obtained. Another reason might be the molecular interactions except electrostatic attractions. Especially for pH 2.5 condition, as well as opposite charges hydrogen-bonding between amine and sulfonate groups in bPEI and NiPcTS respectively govern film growth.

4.3.4. Influence of pH on LbL self-assembly

Another crucial factor influencing LbL film growth is pH of the coating material. By increasing the ionic density in solution or changing the molecular conformation on substrate surface as modifying the pH it is possible to get thicker films by LbL method on any surface that water can diffuse. Effect of pH is highly significant for the control over thin film. Therefore, a set of pH values are studied for NiPcTS and their corresponding outcomes on thin film properties are analyzed.

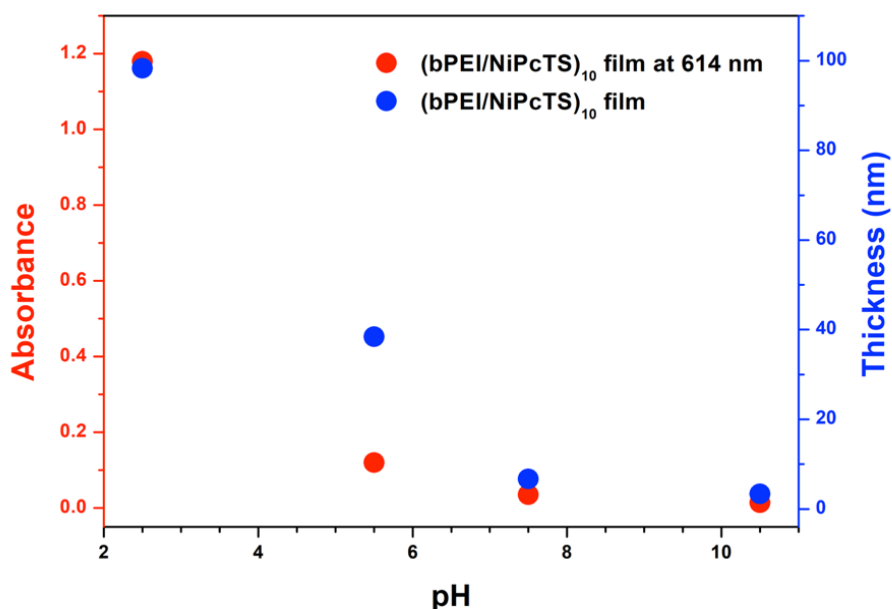


Figure 4.7. Effect of pH on LbL thin film growth for 0.25 mM NiPcTS embedded coating (bPEI/NiPcTS)₁₀

As given at [Figure 4.7](#) highest absorbance is achieved at acidic pH. Increasing pH of the 0.25 mM aqueous NiPcTS solution in (bPEI/NiPcTS)_n film system decreases the absorbance indicating thinner film formation at basic pH values. It is approved by the decrease in film thickness as well. Hence, it is determined that as NiPcTS is dissolved in acidic medium in addition to the effect of ionic distribution, densely loopy molecular conformation contributes to layer-by-layer self-assembly. In other words, once the NiPcTS pH is set to basic due to the collapsed molecular conformation thickness of the film decreases. Thus, control over film thickness is gained by pH of the medium.

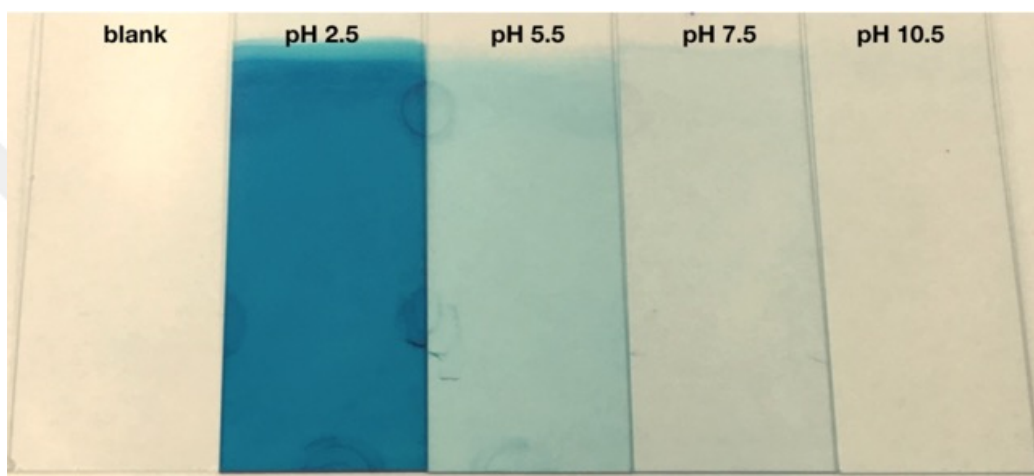


Figure 4.8. Film growth behavior of 0.25 mM NiPcTS containing 10 bL film

As pointed out in the sections above thin film features are clearly monitored as pH of the NiPcTS solution is altered. In [Figure 4.8](#) the dramatic change in color towards light blue is shown for 10 bilayers of (bPEI/NiPcTS) film where NiPcTS is 0.25 mM. Besides, deposition procedure at pH 2.5 is simultaneously monitored by quartz-crystal microbalance on gold coated quartz sensor. To avoid blocking of pipe, concentration of NiPcTS is decreased to 0.01 mM and 3rd frequency tone is used for analysis. [Figure 4.9](#) gives the change in frequency with respect to the number of layers coated to the surface of crystal. To enhance initial interactions, one bilayer of adhesion (PAH/SPS) is applied on crystal surface. Following that, 4 bilayers (4bL) of (bPEI/NiPcTS) are deposited. As given on [Figure 4.9](#) addition of each NiPcTS layer decreases frequency significantly, while bPEI contribution is lesser. This attitude can be addressed to NiPcTS which cause bPEI layer to collapse. A similar trend is observed for repeating layers.

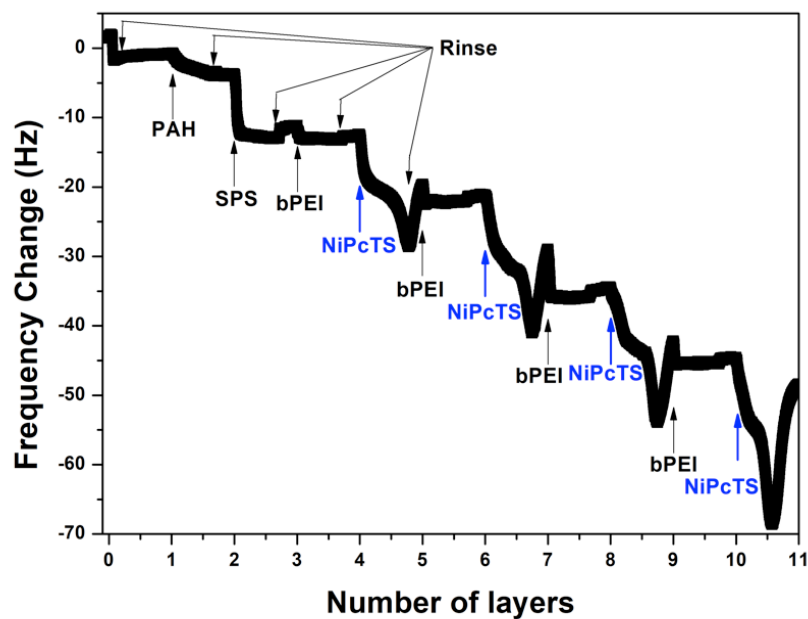


Figure 4.9. Change in frequency for 0.01 mM NiPcTS deposited 4 bilayer (bL) film

Table 4.1. Summary of studied $(bPEI/NiPcTS)_n$ film systems with different pH-concentration values for NiPcTS and the corresponding thickness behavior (PE; Polyelectrolyte, ie. PAH, SPS, bPEI)

	Concentration (mM)	pH	Thickness (nm) for (bPEI/NiPcTS)				
			10 bL	8 bL	6 bL	4 bL	2 bL
PE	10	4.00					
NiPcTS	0.1	2.50	80.9	37.3	18.8	3.5	0.5
		5.50	26.6	17.3	9.6	2.9	-
		7.50	5.8	3.3	1.4	-	-
		10.50	2.7	0.8	-	-	-
	0.25	2.50	98.3	54.2	22.8	8.8	4.4
		5.50	38.4	26.8	14.6	6.4	3.2
		7.50	6.7	5.1	2.6	-	-
		10.50	3.4	1.5	-	-	-

As a summary of studied assembly conditions and their influence on thin film thickness is presented in [Table 4.1](#). In addition to adhesion pair, coating materials are listed in terms of their concentration, pH and corresponding thickness values. Hence, it can be concluded

that 0.25 mM NiPcTS in acidic pH exhibits ~100 nm overall film thickness, while increase in pH to 10.5 decreases film thickness by ~22 fold. Once the phthalocyanine concentration is decreased by 2.5 fold, overall film thickness decreases as well. However, decay in thickness is not linearly dependent on concentration due to the reasons explained above. For some of the cases, where the thickness is shown by a dash, multilayer film coatings are thinner than the equipment can measure.

4.4. Conclusion

Homogeneous and uniform multilayer films of bPEI/NiPcTS are prepared by layer-by-layer self-assembly. Due to the diffusive LbL deposition mechanisms, exponential film growth is obtained at both 0.25 mM and 0.1 mM NiPcTS concentrations. Crucial effects of NiPcTS pH on film growth are reported by absorbance and thickness measurements. It is clearly shown that NiPcTS in acidic media exhibits thicker films with bPEI. Decrease in frequency by NiPcTS addition indicates molecular buildup on the crystal surface. However, instead of pure electrostatic attraction, hydrogen bonding or other additional interactions are taking place for multilayer film formation.

5. Phthalocyanine-based Multilayer Thin Film Coatings for Corrosion Protection

5.1. Introduction

Chemical vapor deposition (CVD) and Langmuir-Blodgett assemblies are two early examples for controlled fabrication of nanostructured thin film assembly methods. In 1970's Kuhn developed composite films from organic molecules with distinctive forms in nanosize⁹⁶. Following that, self-assembly method for organic molecules with functional groups is evolved⁹⁷⁻¹⁰². Nonetheless it has been challenging to generate stable multilayers with controlled thicknesses by Langmuir-Blodgett (LB) approach. As an alternative Layer-by-Layer (LbL) technique has taken the attention of many researchers with its conformal coating on any surface. Superiority of layer-by-layer system arises from its low-cost, simple, environmental-friendly and water-based features. It allows creating multilayer films with nanosize thickness control, conformally and homogeneously any surface where water can penetrate. Oppositely charged molecules in their aqueous solutions are electrostatically attracted by each other to form multilayer thin film coatings. In summary, on top of a negatively charged glass substrate aqueous solution of a positively charged molecule such as polymer, nanoparticle, protein, virus etc. is coated and change of the charge of surface is obtained¹⁰³⁻¹⁰⁹. This cycle lasts until the desired film thickness and morphology are achieved. By using this method polymers, small organic molecules, inorganic compounds^{17, 86-87}, bio macromolecules^{83-85, 110-111} can be embedded to the film system. Fabricated multilayer thin films can be further post-processed for their final applications and films serve for corrosion⁹⁰⁻⁹², antibacterial^{89, 112-113}, anti-reflective⁸⁹ purposes or act as chemical and biological sensors^{88, 114}.

The corrosion resistant activity of poly (diallyl dimethyl ammonium chloride)-PDAC/ poly (styrene sulfonate)-PSS multilayers on stainless steel 316L is studied. 10mM concentrated polyelectrolytes are deposited from 0.25 M NaCl containing aqueous solution and 70 nm thickness is acquired with 20 bilayers. An improvement on corrosion current density by 3.5-fold is reported¹¹⁵.

There are few studies on LbL systems containing controlled phthalocyanine layers. Thin films of copper phthalocyanines serve for solar cell applications⁵⁹, photovoltaic devices¹¹⁶ etc. Corrosion inhibition is another application area for metal-centered

phthalocyanines. To the best of our knowledge, this is the first study in the literature that reports diverse multilayer architectures that could provide protection from corrosion by LbL deposited thin films of polyelectrolytes and phthalocyanines. The corrosion caused by the effect of singlet oxygen shortens lifetime of metals and impair their quality. In the presence of singlet oxygen these metal-centered compounds (phthalocyanines) face with highly reactive oxygen and slow down the corrosion of the metal surface that they are attached to.

There is a need for coatings that will reduce or even eliminate the extremely reactive singlet oxygen-induced corrosion that airborne vehicles are particularly exposed to in the atmosphere. The performance of the thin film coatings prepared here was tested by fundamental electrochemical measurements under atmosphere-mimicking electrolyte conditions. In this study, dip-spin layer-by-layer method is used to fabricate anticorrosive multilayer thin films. Water soluble copper phthalocyanine-3,4',4'',4'''-tetra sulfonic acid tetra sodium salt (CuPcTS) and Nickel(II)phthalocyanine-tetra sulfonic acid tetra sodium salt (NiPcTS) molecules are embedded separately into oppositely charged poly (diallyl dimethyl ammoniumchloride and poly (sodium 4-styren sulfonate) polyelectrolyte layers by LbL method. It is targeted to obtain homogeneous, uniform and stable multilayers. Once appropriate film growth is acquired it is expected to prevent metals from losing their valence electrons in the presence of the atmosphere simulated medium, dilute Harrison's solution (0.35 wt. % $(\text{NH}_4)_2\text{SO}_4$ + 0.05 wt. % NaCl)¹¹⁷⁻¹¹⁸. Corrosion resistant films are developed by combination of polyelectrolyte multilayers and contribution of phthalocyanine layers.

5.2. Experimental Work

5.2.1. Materials

Copper phthalocyanine-3,4',4'',4'''-tetrasulfonic acid tetra sodium salt (CuPcTS) and Nickel(II)phthalocyanine-tetra sulfonic acid tetra sodium salt (NiPcTS) are used as anionic phthalocyanine molecules and Poly (sodium 4-styren sulfonate) (SPS, Mw= 70.000) is used as anionic polyelectrolyte. As polycation Poly (diallyl dimethyl

ammonium chloride) (PDAC, $M_w = 100.000\text{--}200.000$) and Poly (allylamine hydrochloride) (PAH, $M_w = 58.000$) are used and all chemicals are purchased from Aldrich. Distilled water is the solvent of all materials. Both NiPcTS and CuPcTS are water-soluble. The chemical structures of polyelectrolytes and phthalocyanines are shown in [Figure 5.1](#).

The concentrations of phthalocyanines and polyelectrolytes were adjusted to 1mM and 10mM respectively, which are based on the molecular weight of the repeating unit. pH values of polyelectrolytes vary between 4.00-5.50 for PDAC, PAH and SPS. NiPcTS and CuPcTS have intrinsic pH values of 4.50 and 10.70 respectively. All polyelectrolytes and phthalocyanines are purchased from Sigma-Aldrich and used without any purification.

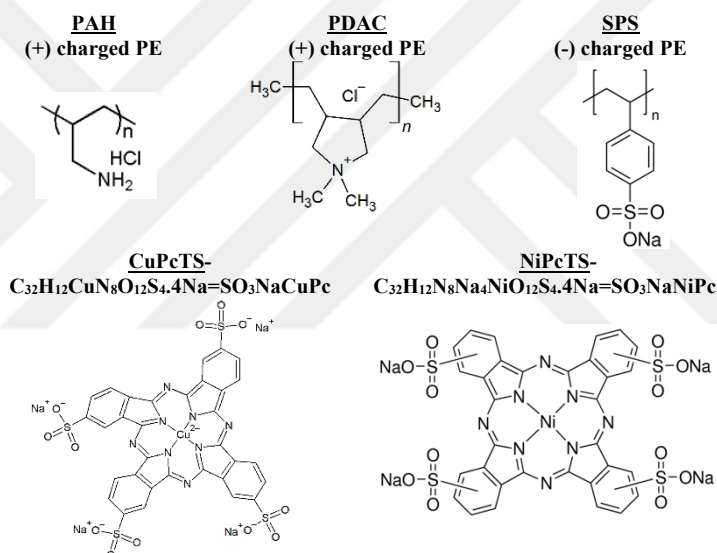


Figure 5.1. Chemical structures of polyelectrolytes and phthalocyanines

5.2.2. Preparation of Layer-by-Layer Films

Layer-by-layer deposition is initiated with PAH/SPS polyelectrolyte pair which are assigned as adhesive layers. Substrates are dip-spin coated with PAH solution of 10 mM, pH 4.20 for 10 min at 100 rpm and rinsed with three different distilled water baths for 2/1/1 min respectively. Following that substrates are immersed into 10 mM SPS solution for 10 min and rinsed again with three distilled water baths. This cycle makes a one-bilayer (bL) film and it is repeated five times to obtain five bilayers of PAH/SPS. Once the substrates are dip-spin coated with PAH/SPS the same coating procedure is followed

30 times for PDAC/ NiPcTS or PDAC/ CuPcTS multilayers giving 30bL of phthalocyanine containing film. Overall film structure is shown in [Figure 5.2](#).

Besides, bilayer film structure and tetra-layer film forms are examined and the contribution and the distribution of phthalocyanine in the film structure are also tested, as well. In [Table 5.1](#), studied layer-by-layer thin films are given.

Table 5.1. Examined film systems each coated with (PAH/SPS)₅ adhesion layers before phthalocyanine multilayer contribution

Sample Name	Phthalocyanine/PE Multilayers	Number of Pc Layers
Bilayer Thin Film Architectures		
S1: PE film	(PDAC/SPS) ₃₀	0
A1: NiPc film	(PDAC/NiPcTS) ₃₀	30
A2: CuPc film	(PDAC/CuPcTS) ₃₀	30
Tetra-layer Thin Film Architectures		
T1: NiPc film	[(PDAC/SPS) ₁ (PDAC/NiPcTS) ₁] ₁₅	15
T2: CuPc film	[(PDAC/SPS) ₁ (PDAC/CuPcTS) ₁] ₁₅	15
P1: NiPc film	[(PDAC/SPS) ₄ (PDAC/NiPcTS) ₁] ₆	6
P2: CuPc film	[(PDAC/SPS) ₄ (PDAC/CuPcTS) ₁] ₆	6

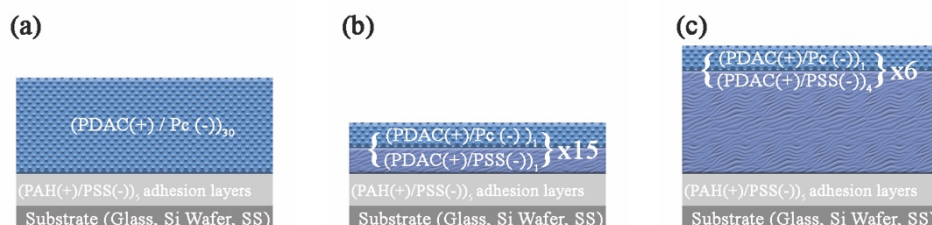


Figure 5.2. Film morphology achieved by LbL method (a) bilayer, (b) and (c) tetralayer LbL Film Structure

5.2.3. Characterization

Thickness of layer-by-layer films on glass plates and Si-wafers are measured with KLA Tencor P6 profilometer and Woolam M2000 ellipsometry respectively. Thin film growth behavior is also monitored by Cary 5000 UV-VIS-NIR spectroscopy in the visible range. All electrochemical measurements such as Tafel, potentiostatic impedance etc. are performed with PARSTAT MC potentiostat. Gamry Corrosion Paintcell with diluted

Harrison's solution (0.35wt% $(\text{NH}_4)_2\text{SO}_4$ + 0.05wt% NaCl) is the electrolyte of each electrochemical measurement which provides almost the same effect of atmosphere for aerospace vehicles. In terms of reference electrode and counter electrode Ag/AgCl in saturated NaCl solution and graphite rod are preferred respectively. Working electrode area is 1 cm^2 and all measurements are performed under room temperature.

Before each potentiostatic impedance spectroscopy measurements metal sample is immersed into the Harrison's solution for 1 hour. The open circuit potential is balanced, and the impedance measurement is performed between 10^5 -1 Hz with 10mV amplitude.

5.3. Results and Discussion

5.3.1. LbL Film Characterization

Thickness of the layer-by-layer films is examined by profilometer and ellipsometry measurements. Alternatingly coated polyelectrolyte and phthalocyanine layers do interact electrostatically. This interaction results in the linear growth of film thickness. Optically, color change of the films from lighter blue to darker indicates film growth as well. According to the measurements performed by KLA-Tencor profilometer growth curves are presented in [Figure 5.4](#).

Digital images of 0.01mM aqueous phthalocyanine solutions are given in [Figure 5.3](#). Both NiPcTS and CuPcTS have blue color. Once they are transferred to stainless steel surface ([Figure 5.3b](#)) NiPcTS containing 30bL film (A1) presents an intense blue color than CuPcTS coated film (A2). Nickel phthalocyanine films show a more dramatic change in color towards dark blue while copper phthalocyanine films are pale and thinner once they are spin-dip coated. As an indication of homogeneous distribution of the phthalocyanines in the films, homogeneous color change was observed during film growth. Change in color is monitored by absorbance measurement in the visible range. It is expected to have an increase in absorbance as the number of layers increases.

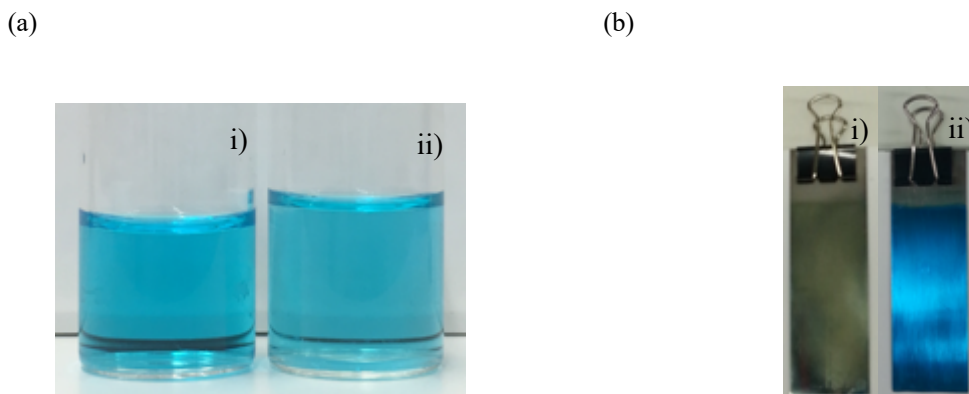


Figure 5.3. Digital images of (a) aqueous solutions of i) 0.01 mM CuPcTS and ii) 0.01 mM NiPcTS, (b) multilayer films of i) A2; CuPcTS and ii) A1; NiPcTS structures on 304 stainless steel

Taking advantage of the colorful appearances of NiPcTS and CuPcTS, multilayer thin film growth is investigated by UV-vis spectroscopy. Absorbance spectra of aqueous and solid phases for both phthalocyanine conditions are compared in [Figure 5.4a](#). Both phthalocyanine molecules give maximum absorbance around 606 nm as presented by dashed curves for 0.01mM phthalocyanine solutions and by solid curves for 10mM phthalocyanine embedded 30bL films. Because of the transition between π - π^* interaction, Q-band of CuPcTS and NiPcTS are clearly shown by the strong absorbance peak around 606 nm.

Film growth in terms of absorbance of all studied coatings are given in Figure 4b. Maximum absorbance values, which arise at 606nm for all tetralayer structures are plotted with respect to the number of tetralayers. Similarly, inset of [Figure 5.4b](#) represents the same property for bilayer films only. Absorbance maxima are 610nm for A1 and 606 nm for studied bilayer coatings. According to [Figure 5.4b](#) it is determined that tetralayer structures exhibit an exponential growth as the number of layers increases while the bilayer structures show a linear growth. This might be due to the increase in polyelectrolyte concentration in the multilayer film system. While bilayer coating structures contain 30 layers of polyelectrolyte only, the number of polyelectrolyte layers increases in tetralayer structures. Swelling or diffusion of PDAC into previously coated layer may lead to indistinct layer distribution.

Multilayer film growth is also examined by change in overall film thickness as given in [Figure 5.4c](#). Similar to the growth behavior obtained by absorbance spectra, bilayer films

provide almost linear trend while tetralayer structures have an exponential increase in thickness as the number of layers increases.

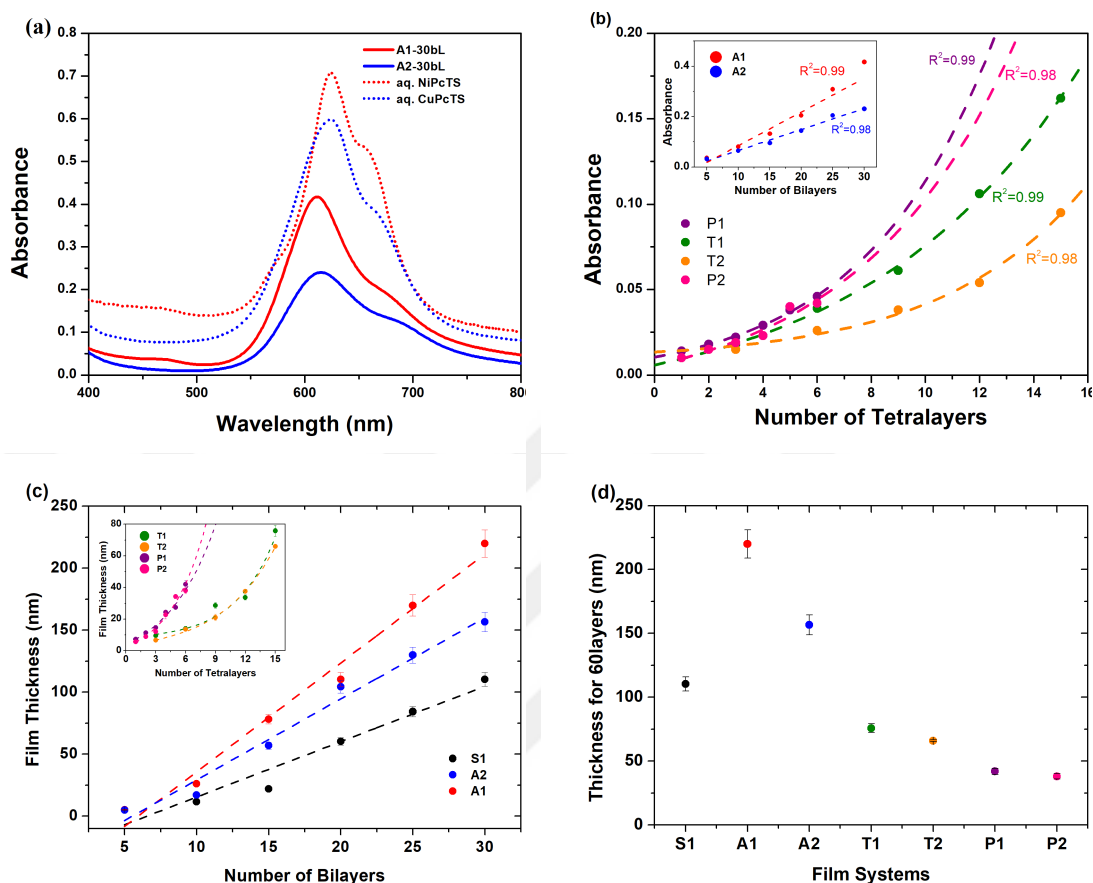


Figure 5.4. (a) UV-vis absorbance spectra of 0.01mM aqueous (--- dashed curve) and solid films (— solid curve) of A1 and A2 coating systems, (b) Film growth in terms of absorbance with respect to the number of tetralayers at 606nm; inset figure represents with respect to the number of bilayers at 610 nm (A1) and 606 nm (A2) respectively, (c) Film growth in terms of thickness with respect to the number of bilayers; inset figure represents with respect to the number of tetralayers, (d) Comparison of thicknesses obtained for the 60-layer thin films of all film architectures.

Film thickness of adhesion layers (PAH/SPS) is obtained as ~ 0.4 nm/bL. The thickness values of phthalocyanine containing bilayer films were measured as 7.3 nm/bL for A1 and 5.2 nm/bL for A2. Tetralayer (tL) distribution of phthalocyanine represent thickness between 5.0-4.4nm/tL for T1-T2 and 6.9-6.4nm/tL for P1-P2 systems respectively. The polyelectrolyte pair PDAC/SPS has an average thickness of 3.6 nm/bL. As the substrate is coated by 30 bilayers of PDAC/NiPcTS the thickness of films obtained as to be 220 nm. Among all studied film systems, copper containing phthalocyanine complexes produce thinner films than films including nickel-centered molecules. Thin films with copper phthalocyanine layers have a pale solution color is the same for both NiPcTS and

CuPcTS as given on [Figure 5.3b](#). As the contribution of NiPcTS in the coating system decreases by 5-fold (P1) overall film thickness decreases, from 220nm (A1) to 42nm (P1), almost by 5-fold as well ([Figure 5.4d](#)). Similar trend is also observed for CuPcTS containing film system.

5.3.2. Corrosion Behavior

Electroactivity of phthalocyanines containing multilayer films are examined by cyclic voltammetry. CV of bare polyelectrolyte coated LbL structure S1 is used as a reference plot. Both single scan and multiple scan graphs are given for polyelectrolyte coated S1, bilayer and tetralayer forms of NiPcTS, CuPcTS as A1, A2 and P1 respectively. Since S1 does not contain any electroactive component, it is expected to obtain flat graph. When phthalocyanine molecules are introduced to the coating system, electroactivity appears clearly. Redox peaks are distinguishable for bilayer NiPcTS and CuPcTS coated films. However, only oxidation is taking place for the tetralayer structure P1. Besides, the presence of intense anodic peak compared to the cathodic side indicates an irreversible mechanism.

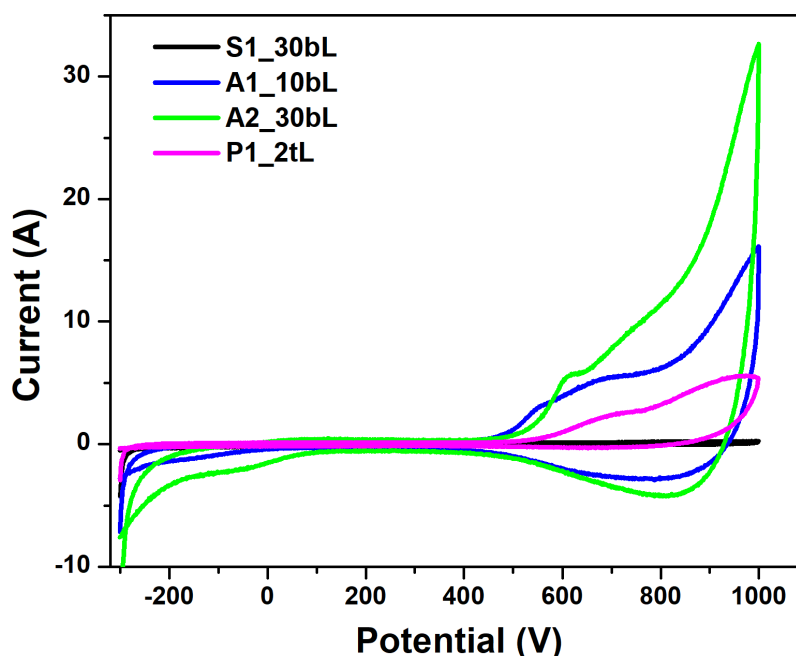


Figure 5.5 Single scan cyclic voltammery of bilayer and tetralayer coatings

According to multiple scan CV curves, current density is decreasing for each phthalocyanine introduced system. The reason behind can be that the films might not be electrochemically stable enough to preserve their redox behavior.

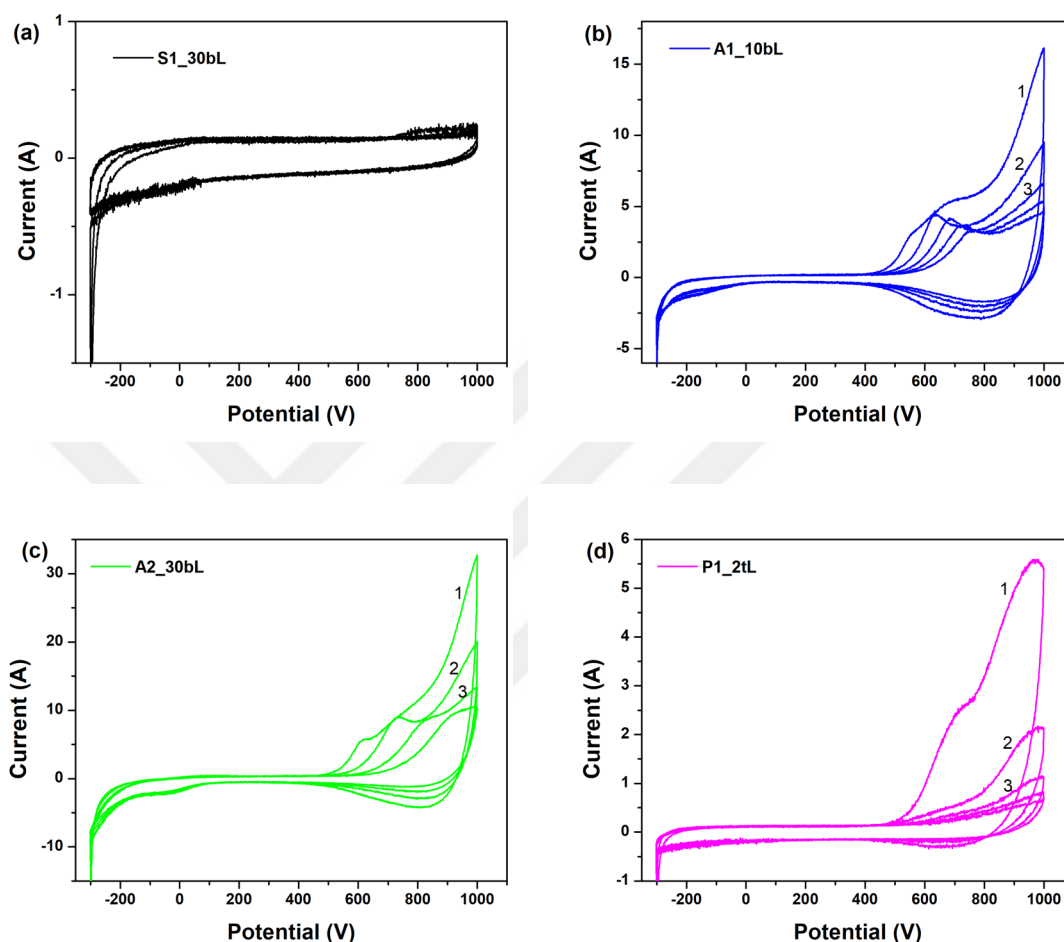


Figure 5.6 Multiple scan cyclic voltammetry graphs of polyelectrolyte and phthalocyanine coated films

The electrochemical properties of layer-by-layer films are analyzed by Tafel measurement and potentiostatic impedance (capacitance) spectroscopy. According to potentiostatic impedance measurements complex impedance, complex capacitance and shift in phase angle with respect to frequency change are determined.

All film systems listed on [Table 5.1](#) are prepared on stainless steel (SS) 304. In order to compare the effect of phthalocyanine density in the film system on corrosion behavior SO_3NaNiPc -Nickel(II)phthalocyanine-tetra sulfonic acid tetra sodium salt and SO_3NaCuPc -Copper phthalocyanine-3,4',4'',4'''-tetra sulfonic acid tetra sodium salt are

distributed among PDAC and SPS polyelectrolyte layers (film architectures of T1, T2, P1 and P2). Once homogeneous and uniform layers are established their corrosion rates are calculated from Tafel curves according to the constants of metals in literature and the corrosion tendency is estimated by transforming complex impedance plane ($Z^* = Z' + jZ''$) to complex capacitance plane (C^*), where conventional Nyquist remain incapable¹¹⁹⁻¹²⁰.

$$\text{Corrosion Rate (mpy)} = \frac{I_{corr} \times K \times EW}{d} \quad (5.1)$$

- I_{corr}** : Corrosion current density ($\mu\text{A}/\text{cm}^2$)
 K : Corrosion constant ($1.288 \times 10^5 \text{ milli-inches}/(\text{A-cm-year})$)
 EW : Equivalent Weight (g)
 d : Density of the metal used (g/cm^3)

$$C^*(\omega) = C'(\omega) - j.C''(\omega) \quad (5.2)$$

- C'** : Real component of complex capacitance plane
 C'' : Imaginary component of complex capacitance, where $j^2 = -1$
 ω : Angular frequency
 Z' : Real component of complex impedance plane
 Z'' : Imaginary component of complex impedance plane

$$C'(\omega) = \frac{Z''(\omega)}{\omega \cdot |Z(\omega)|^2} \quad (5.3)$$

$$C''(\omega) = \frac{-Z'(\omega)}{\omega \cdot |Z(\omega)|^2} \quad (5.4)$$

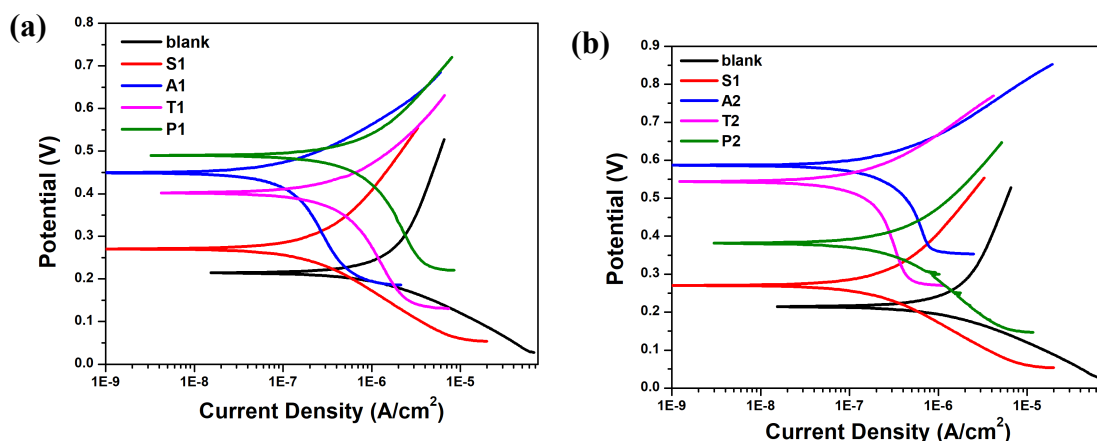


Figure 5.7. Current density vs potential diagram for (a) NiPcTS and (b) CuPcTS film systems on stainless steel 304

The corrosion investigations of multilayer thin film coatings on SS 304 are given as Tafel curves in [Figure 5.7](#) and values calculated from these curves are summarized in the [Table 5.2](#). In the presence of Nickel phthalocyanine thin film coated A1 architectures onto SS 304 substrates almost 10 folds of lower corrosion rates are observed. As metal-centered compounds are introduced to the film structure corrosion potential rises to 0.45V from 0.21V. It is even greater for the copper phthalocyanine film of A2. Besides, the corrosion current density of stainless steel 304 coated with A1 film system is 10 times lower than the blank stainless steel. Although corrosion potential and corrosion current densities of tetra-layer structures (T1/P1/T2/P2) are shifted to more noble regions improvement corrosion rates remains insignificant for protection compared to the polyelectrolyte paired film (S1) without phthalocyanine.

On 304 stainless steel the corrosion rate of A1 system arises to be 0.07 mpy which is almost equal to expected rate (~ 0.065) when 60 layers polyelectrolyte coated S1 system is taken as reference. Although the corrosion rate of A2 system is 2.5 times higher than the expected rate, A1 satisfies the required condition with lower corrosion rate compared to S1 and blank 304 stainless steel. Since tetralayer structures contain less phthalocyanine layers than bilayer systems it is expected to get closer corrosion rate values to completely polyelectrolyte containing S1 system. Among tetralayer structured systems T2 gives the most consistent corrosion rate with 0.12 mpy. T1 with 15 layers of Nickel phthalocyanine

in the total system has ~3.6 higher corrosion rate than calculated. For further investigation impedance spectroscopy measurements are performed under the same electrolyte condition.

Table 5.2. Corrosion data of stainless steel 304 for different coating architectures

Sample Name	Corrosion Potential (V)	Corrosion Current Density ($\mu\text{A}/\text{cm}^2$)	Corrosion Rate (mpy)
304SS_blank	0.21	1.91	0.75
304SS_S1	0.27	0.32	0.13
304SS_A1	0.45	0.19	0.07
304SS_T1	0.40	0.89	0.35
304SS_P1	0.49	1.68	0.66
304SS_A2	0.59	0.41	0.16
304SS_T2	0.54	0.31	0.12
304SS_P2	0.38	0.52	0.20

In the present study, complex impedance plot (Z^*) give almost vertical lines, [Figure 5.8](#), where the interface is expressed by circuit elements (RC-resistor, capacitor) in series. There is an indication of strong capacitive behavior according to the Z plots of studied thin film systems. Therefore, complex capacitance plane (C^*) is preferred to obtain almost complete semicircles. Imaginary part of complex capacitance is associated to conductivity and increases as the number of nickel phthalocyanine content in multilayer film is decreasing. Tetralayer thin film distribution (P1 & T1) of NiPcTS display higher conductivity among all studied film systems. Towards low frequency region, imaginary part of capacitance diverges from zero, displaying a slow mass transport process¹²⁰. Furthermore, maximum NiPcTS concentration containing film system (A1 with 30layer of phthalocyanine) exhibits a faradaic process in the mid-low frequency region and the calculated total capacitance, which is obtained from the intercept of -Im capacitance with real axis, is lower compared to other film systems. It might be because of the saturated phthalocyanine concentration and density among multilayers, since A1 is the only bilayer system with maximum number of nickel phthalocyanine content.

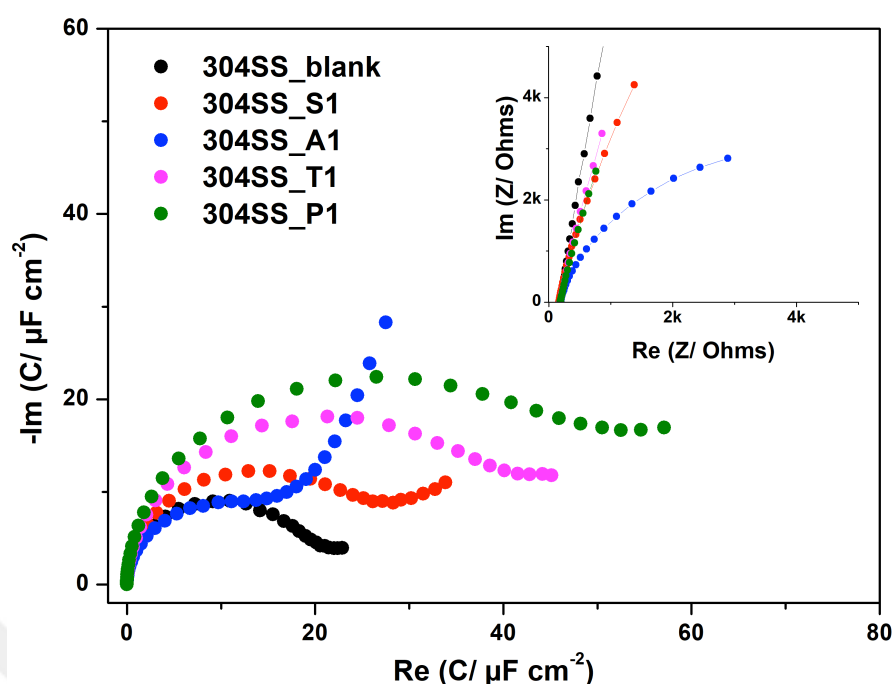


Figure 5.8. Complex capacitance and complex impedance (in the inset) of (NiPcTS) multilayer thin film coatings on stainless steel 304.

In order to interpret vertical complex impedance behavior of studied film systems, impedance magnitude $|Z|$ and phase angle with respect to frequency are plotted as well, [Figure 5.9](#). While filled circles ($\bullet$) represent impedance modulus, unfilled circles ($\circ$) stand for phase angle shift of impedance. Even before any ozone treatment, blank stainless steel has the highest phase angle with 80° . There is a significant decrease to 43° when the metal is homogeneously coated by 30bL of NiPcTS film. The reason behind the sharp decrease is attributed to the higher ion conductivity¹²⁰ in the system, since A1 carries maximum phthalocyanine concentration by 30 bilayers. Hence, it is expected obtain a similar feature for A2 coating, which contains 30 bilayers of copper phthalocyanine. As predicted, phase angle shifts from 80° (blank) to 44° for A2 coded multilayer thin film (data not given). Although, the rest of the studied film systems do not have a significant shift in phase angle compared to blank 304 stainless steel, their conductive character is obvious in the low frequency region by a short decline ($\sim 5^\circ$) in phase angle. Above 1k Hz all coatings

show resistive character with 0° phase angle and below 100Hz slope of the Bode plot ($|Z|$ vs freq.) approaches to -1 demonstrating capacitive nature.

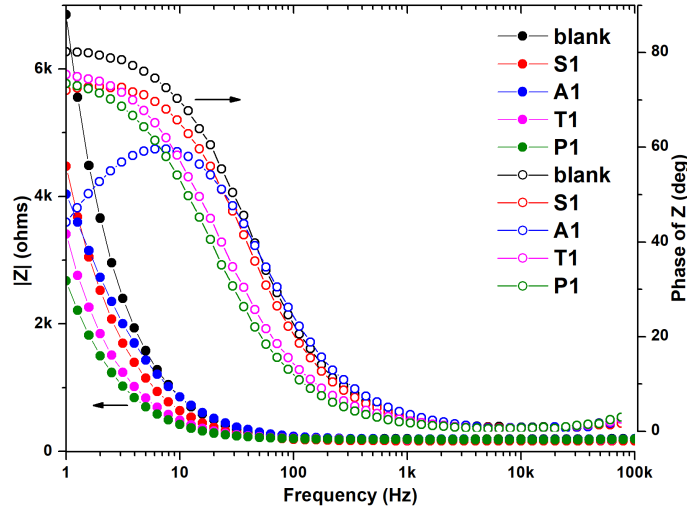


Figure 5.9. Bode plots of (NiPcTS) bilayer and tetralayer thin film coatings on stainless steel 304

Frequency dependency of complex capacitance parts is used to determine relaxation time constant $\tau(1/2\pi f_0)$ and static capacitance thorough imaginary and real capacitance respectively¹²¹. Time constant in the present case is in the order of $\mu\text{F}/\text{cm}^2$ and presence of any coating on metallic surface activates the capacitive feature at lower frequency. Investigated resistive behavior at high frequency region at [Figure 5.9](#) is supported by the horizontal trend at high frequency in [Figure 5.10](#). It is noticed that the tetralayer structure P1 has the highest relaxation time or i.e. lowest response frequency (f_0) indicating better energy delivering supercapacitive coating property¹²²⁻¹²³.

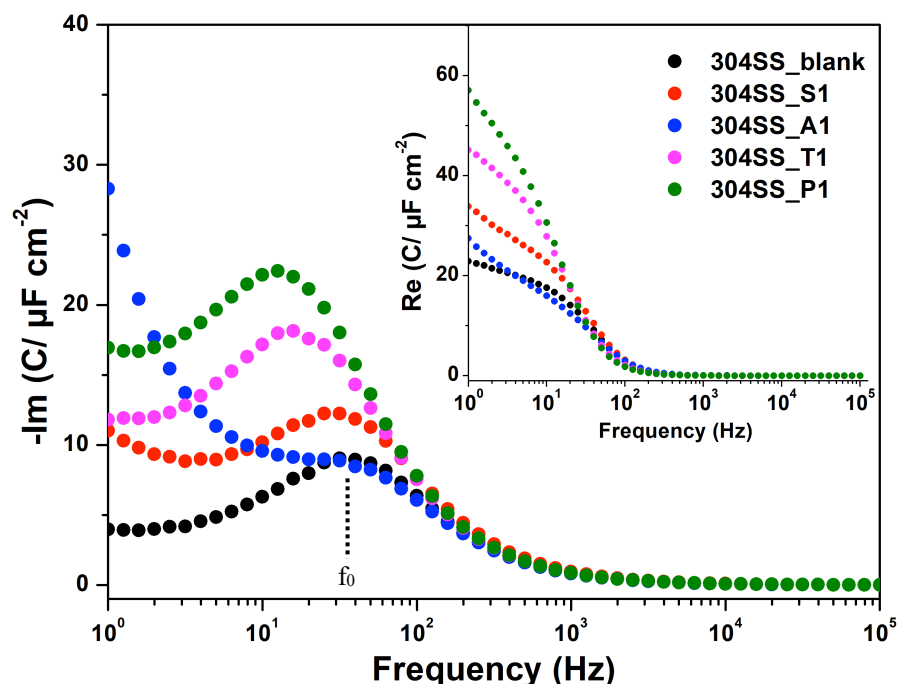


Figure 5.10. Frequency dependency of NiPcTS containing multilayer thin films on stainless steel 304 surface

By extrapolating the semicircles obtained at [Figure 5.8](#) at low frequency end, total capacitance of captured ions inside multilayer thin film is determined. The intercept of imaginary part of capacitance up to mid-frequency with the real capacitance is estimated as the semicircle diameter and plotted with respect to the number of phthalocyanine layers in multilayer thin film, i.e. studied film systems.

It is observed that the coating capacitance of the metal 304SS increases in the presence of any film system. According to [Figure 5.9](#), maximum phase degree of 30bilayer A1 film structure has a phase shift towards higher frequency due to the slow current response¹²⁴. The reason behind could be the high charge transfer resistance of the coating that delay current response. Capacitance is dependent on the thickness of coating inversely¹²⁵. A 30-bilayer film system with Nickel and Copper phthalocyanines have the highest thickness among all systems. Therefore, it is expected to see smallest capacitance for A1 and A2. Once the calculated coating capacitance values are compared, P1 film system gives highest capacitance values for 304SS.

The significant changes are observed with the films that contain more polyelectrolyte than the phthalocyanines. Please note that S1 has the highest amount of polyelectrolyte and SPS contains aromatic moiety which is expected to exhibit different decomposition pattern. However, film resistance values of T1 and P1 films are quite high compared to all studied coatings which gives the hint that they will exhibit much better corrosion protection.

5.4. Conclusion

Phthalocyanine based multilayer films of PDAC/NiPcTS and PDAC/CuPcTS were fabricated by layer-by-layer spin-dip deposition technique. The assembled films were homogeneous, and uniform distribution of phthalocyanines was observed within the films. The thickness of the films was controlled in the order of nanometers and growth behavior is reported. While tetralayer film structures exhibit exponential growth, bilayer multilayer films grow linearly. The electrochemical properties were investigated, and it is shown that metal substrate coated with the polyelectrolyte film protects the surface more compared to the blank metal. Towards low frequency end multilayer thin films exhibit a dominant faradaic process. Decrease in phase angle towards low frequency end for phthalocyanine containing bilayers is attributed to higher ion conductivity. Although 30bL phthalocyanine containing multilayer coatings contain higher phthalocyanine amount within the film, distribution of these capacitive layers is significant as well. It is concluded that not only the phthalocyanine concentration but also their distribution throughout the coating system play a critical role. Consequently, tetralayer architectures T1 and P1 exhibit higher capacitive characters.

6. Multilayer Thin Films of Zinc (II) phthalocyanine Loaded Poly(D,L-lactide-co-glycolide) Nanocapsules by Layer-by-Layer Self-Assembly

6.1. Introduction

More than twenty thousand articles published every year for the delivery of chemically active materials and their controlled release mechanism in recent years. Since the performance of drug delivery systems is strongly dependent on drug loading and releasing features, there is still a continuous need for advanced technologies to transfer various active drug materials to the target tissue. Layer-by-layer (LbL) self-assembly method has been taking attention of many researchers as an alternative to nanostructured film fabrication techniques present in the literature, such as chemical vapor deposition (CVD) or Langmuir-Blodgett (LB) films. Since 1970's there is ongoing research for conventional, unconventional and quasi-LbL approaches^{21, 43, 126}. The convenience of LbL technique arises due to its simple, water-based, environmental-friendly, versatile aspects and it is easily applicable to any surface that water can penetrate. Additionally, precise control over material quantity and film thickness in nanometer scale can be established¹²⁷. In LbL, multilayer thin film formation is obtained by electrostatic attraction of oppositely charged materials^{103-104, 127-128} such as polyelectrolyte, protein, nanoparticle, virus, etc. in aqueous media and thin film behavior can be modified by changing temperature, pH, ionic strength or concentration of the coating material. Fabricated thin films serve for several applications like anti-reflective, anti-bacterial or biosensor¹²⁹ purposes. Especially in the last decade LbL approach has been studied for various biomedical applications¹³⁰⁻¹³⁴, drug delivery purposes¹³⁵⁻¹³⁷, biomedical devices¹³⁸ through self-assembly of LbL technique. Desired drug, molecule or nanoparticle can be surrounded by polymeric carriers, which are formed through encapsulation of the active molecule by LbL approach. Another process in the literature is to encapsulate the active molecule by using the hydrophilic/hydrophobic¹³⁹⁻¹⁴⁰ interaction relationship between drug and copolymer. Stimulation by pH¹³⁰, charge¹³⁹ or temperature¹⁴¹ are most common mechanisms used to load and release drugs. Multilayer thin films of hydrophobic molecules^{139, 142-143} prepared by LbL approach provide precise control of coated material on the surface both quantitatively and morphologically.

Photodynamic therapy (PDT) is a combined cancer treatment method requiring a photosensitizer molecule, light to activate the photosensitizer and oxygen to generate tumor destroying reactive singlet oxygen¹⁴⁴⁻¹⁴⁵ with numerous advantages¹⁴⁶ reported in the literature. PDT is a repeatable process without any surgical operation, positively tolerated especially at early stage cancer and increases the life quality period of patients. Novel studies performed within wearable patches and bandages compatible with PDT offers a positive alternative where surgery is not possible and/or enough. Within highly tailorable characteristics of photosensitizers, unique drug combinations can be studies to suit each patient. An ideal photosensitizer should have a high singlet oxygen generation quantum yield ($\Phi\Delta$) and should absorb light between 650-800 nm for maximum light penetration through tissue^{82, 144}. There are several different photosensitizers in the literature and photofrin is one of the clinically approved porphyrin-based photosensitizers¹⁴⁵. Since photosensitizers play a significant role in PDT, there is ongoing research for alternative molecules. Zinc (II) phthalocyanine is one of the most promising photosensitizers candidates among second generation photosensitizers^{82, 147}. Due to its molecular structure and photochemical properties, high efficiency against tumor tissue has been declared^{53, 148}. The main advantage of ZnPc lies in its hydrophobic character, which enables high encapsulation efficiency. Liposomes^{62, 149}, polymer micelles¹⁴⁹⁻¹⁵⁰ or nanoparticles¹⁵¹ are commonly preferred as carriers to deliver the photosensitizer to the target tissue. Among several different carriers, poly(D, L-lactide-co-glycolic acid) (PLGA) is the most common carrier within its biocompatibility, biodegradability and non-immunogenic character¹⁵²⁻¹⁵³. Hence, PLGA has also been approved by the Food and Drug Administration (FDA) for therapeutic applications¹⁵³. However, only some of the photosensitizer loaded carriers can arrive to the target, which decreases the desired performance. Besides, it is highly challenging for near-infrared (700-2500nm) or visible (400-700nm) light to penetrate through tissue¹⁴⁶ deeper than 1-3mm¹⁴⁴ while initiating the photodynamic reaction. Considering short lifetime of the generated reactive singlet oxygen, PDT is strongly recommended in the literature for tumors on skin or just under the skin. Hence, a need for the improvement in the direct efficiency of photosensitizers on tumor cells is required to enhance the possibility of using these materials for further drug testing.

The aim of this study is to develop multilayer thin film structures of ZnPc loaded PLGA capsules for future local skin-oriented photodynamic therapy applications with controlled amount and distribution among polyelectrolyte layers. Oppositely charged polyelectrolyte layers and encapsulated hydrophobic photosensitizer are coated by dip-spin layer-by-layer self-assembly and the effect of thin film structure, pH of capsules on multilayer film adsorption and distribution are investigated. In here, an average size of photosensitizer particles is obtained smaller than <100nm that is reported first time in the literature with 86 % yield which is stated as crucial for the efficiency of drug delivery¹⁵⁴. As a scalable and controlled method, LbL offered a uniform distribution of hydrophobic photosensitizers ZnPc loaded PLGA capsules below 10 nm precisely. The developed technology with this work would find use for thin film coatings on absorbable gelatin sponges or microneedle bandages for in-vitro photodynamic applications.

6.2. Experimental Section

6.2.1. Materials

Zinc (II) phthalocyanine (ZnPc, Mw=577.91), poly (D, L-lactide-co-glycolide) (PLGA 50:50, Mn=18.000-32.000), poly (vinyl alcohol) (PVA, Mw=13.000-23.000, 98% hydrolyzed) are used for nanoparticle formation. Poly (sodium 4-styren sulfonate) (PSS, Mw= 70.000) as anionic polyelectrolyte and branched poly (ethyleneimine) (bPEI, Mw= 25.000) as cationic polyelectrolyte are used. Dichloromethane (DCM), ethanol, pyridine (all analytical grade) and all chemicals are purchased from Sigma-Aldrich.

Polyelectrolyte concentrations were adjusted to 10mM based on the molecular weight of repeating unit. pH values of polyelectrolytes are set to 4.00 for bPEI and PSS. All polyelectrolytes, polymer and phthalocyanines are used without any further purification.

Glass slides (75x25x1mm) and Si-wafers (50x15mm) are used as substrates for thickness, roughness, morphology and absorbance measurements by profilometer, scanning electron microscopy (SEM), atomic force microscopy (AFM) and UV-visible spectrometer (UV-Vis) respectively. Substrates are ultrasonicated with Micro-90 concentrated cleaning solution-purchased from Sigma-Aldrich- and distilled water for 20 min each respectively. All substrates are dried with N₂-flow and plasma cleaned for 3-4

min by Harrick Plasma Cleaner. Besides, 5MHz, 14mm diameter AT-cut gold coated sensor crystals are used for quartz-crystal microbalance measurements. Before each measurement, cleaning protocol of Q-Sense is applied. Crystals are UV/ozone treated for 10 minutes in a chamber, in which light is generated at 185 nm and 254 nm wavelengths to remove low molecular weight hydrocarbons through oxidation. A 5:1:1 mixture made of milliQ water, ammonia (25%) and hydrogen peroxide (30%) is heated up to 75°C. Sensor crystal is placed into the heated solution for 5 minutes and rinsed with milliQ water. Crystals are dried under nitrogen gas flow and retreated by UV/ozone for 10 more minutes.

6.2.2. Preparation of Zinc (II) Phthalocyanine loaded PLGA nanoparticles

Encapsulation of phthalocyanines into nanospheres previously published elsewhere^{75, 148, 152} and similar protocol is followed for the current study. Briefly, 100mg PLGA and 0.5mg zinc (II) phthalocyanine are dissolved in 5ml dichloromethane (DCM), and 1w/v % PVA aq. solution is prepared. Organic phase to aqueous phase ratio is 1:2 and organic phase is added to PVA solution dropwise while mixing by IKA Ultra-turrax T18 emulsifier at 20400 rpm. Obtained emulsion is probe sonicated at 50W output for 5min. Organic solvent DCM is evaporated by magnetic stirring under room temperature for 4h. Stabilizer PVA in the emulsion is removed from the system by 15 min centrifugation at 10,000 rpm and resuspended in water. This step is repeated for at least three times until ZnPc encapsulated PLGA nanoparticles are purified. Particles are freeze-dried and stored for characterization.

6.2.2.1. Process Yield

After freeze-drying, nanoparticles are obtained in powder form. The process yield is calculated as in Eq.1:

$$Y\% = \frac{M_{np}}{M_t} \times 100 \quad (6.1)$$

where Y% is the percent process yield, M_{np} is the mass of dried nanoparticles and M_T is the initial sum of mass (PLGA with ZnPc). Process and the percent yield determination is repeated for three times.

6.2.2.2. Percent encapsulation efficiency

Quantification of ZnPc in encapsulated PLGA nanoparticles are measured by Shimadzu UV-3150 UV-VIS-NIR spectroscopy and checked by fluorescence emission spectroscopy with a similar protocol in the literature is followed^{75, 148, 152}. Briefly, loaded nanoparticles in powder form were dissolved in pyridine and 0.5ml of sample is diluted with 2 % SDS buffer saline. After mixing for 2 h at room temperature obtained suspension is centrifuged at 10000g for 15 min. ZnPc content in the collected supernatant is measured by the fluorescence emission spectrum and the area is used to determine the ZnPc amount. Samples are excited at 600 nm and fluorescence emission spectra are measured between 640 and 800 nm. According to standard curve ZnPc concentrations are determined and encapsulation efficiency is calculated from Eq.2:

$$EE\% = \frac{M_s}{M_T} \times 100 \quad (6.2)$$

where EE% is the percent encapsulation efficiency, M_s is the mass of ZnPc in nanoparticles and M_T is the theoretical mass of ZnPc used in formulation.

6.2.3. Multilayer thin film assembly

Layer-by-Layer method is used to deposit thin film coatings on each substrate (glass, silicon-wafer, etc.) and all thin film systems were obtained by dip-spin LbL method. First, negatively charged substrate is immersed and spun in positively charged 10mM bPEI polyelectrolyte solution for 10min with 100rpm, then rinsed in wash bath three times (2/1/1min respectively) and reimmersed in an oppositely charged 10mM PSS polyelectrolyte solution for another 10min and spun at 100 rpm. Excess material on substrate surface is again removed in three different wash baths. This cycle is called as a bilayer and the procedure is repeated for 5 times to obtain 5 bilayers of adhesive multilayers (bPEI/PSS)₅. Lastly, aqueous solution of ZnPc encapsulated PLGA nanoparticles (0.032 mg/mL) is coated on another 10mM bPEI layer for 10 min dipping and spinning by 100 rpm. Excess material is again washed away in three rinse baths. This cycle can be repeated until the desired film structure is obtained.

6.2.4. Characterization

Aqueous solutions of zinc (II) phthalocyanine loaded PLGA nanoparticles are prepared for particle size and surface charge measurements. Malvern instruments DLS Nano-ZS is used for surface charge measurements only. For size distribution and nanoparticle morphology, Zeiss LEO Supra 35VP scanning electron microscopy is used. Samples both in powder and thin film form are coated by Au/Pd target for electron conductivity. Average size is determined by using a computer program Image J for an accurate distribution analysis.

Multilayer thin film growth is determined by the change in absorbance which is monitored by Shimadzu UV-3150 UV-VIS-NIR spectrophotometer. Light absorbance and transmittance of thin films with different number of layers coated on glass slides are investigated with respect to blank glass. Thickness of self-assembled films on glass slides is measured with KLA Tencor surface profiler P-15. Quartz-crystal microbalance measurements are performed by Q-Sense E1 to quantify photosensitizer amount coated on the surface. LbL assembly is applied on 5 MHz, 14mm AT-cut sensor with gold electrodes. Flow rate for each coating material is 0.1 ml/min and frequency and dissipation characteristics are recorded simultaneously with the LbL coating at room temperature. Scanning electron microscopy in secondary electron image mode is used to investigate the surface distribution and morphology of coatings. Surface topography of deposited thin films on Si-wafer is investigated by Bruker MultiMode8 atomic force microscopy in tapping mode. Scan rate is 0.5Hz with 256 sample lines.

6.3. Results and Discussion

6.3.1. Size and size distribution of ZnPc encapsulated PLGA nanoparticles

Average size distribution of encapsulated particles are determined according to measurements performed by scanning electron microscopy. Achieving a successful assembly of multilayer thin films is significantly related to the size of synthesized particles as accomplished in this study. It is highly challenging to prepare homogeneous

coatings of ZnPc loaded PLGA particles when the sizes of them are at in the order of microns by any method. To avoid precipitation of particles, synthesis should be achieved in nanometer scale to form continuous and uniform thin films without any agglomeration. Hence, as a first step organic phase is added on top of the aqueous phase in the presence of homogenizer to reach 1 μ m average particle size as can be seen in [Figure 6.1a](#). Decreasing the average particle size from micrometer to nanometer is performed by probe sonication. The SEM images showed that with a successful sonication the size of the spheres varies between 25nm-163nm ([Figure 6.1b](#)). Particle size distribution is calculated by using Image J and plotted with respect to the average size as shown in [Figure 6.1c](#). It is clearly seen that the majority of the prepared nanoparticles (86.4%) are less than 80 nm, which is significantly smaller than the prepared ZnPc loaded PLGA nanoparticles in the literature (285nm)⁵³. Additionally, percent yield (Y%) for ZnPc loaded PLGA nanoparticles and encapsulation efficiency (EE%) are calculated by equations (6.1) and (6.2) as 86% and 52.3% respectively. Stability of the system is also measured as -25.4 $\pm$ 2.9 mV.

Zinc phthalocyanine possesses maximum light absorption around 675-701 nm depending on the solvent properties. In [Figure 6.2](#), light absorption for standard ZnPc in dichloromethane is measured at 669 nm. After the encapsulation process of ZnPc by copolymer PLGA, maximum absorption is again obtained at the same wavelength. For fluorescence measurements both the standard and encapsulated ZnPc are excited at 640 nm and maximum fluorescence emission intensities for both conditions appear at 674nm which clearly proves that the encapsulation of ZnPc by copolymer PLGA does not interfere its photophysical properties. This is also crucial for future applications of the ZnPc loaded PLGA nanoparticles to be used as photosensitizers in a wearable patch to investigate the effectiveness in PDT.

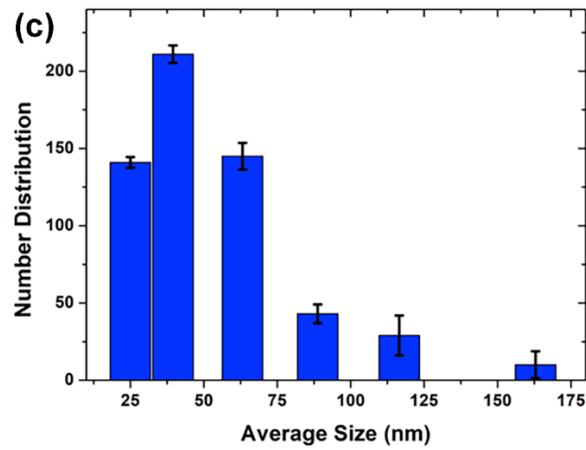
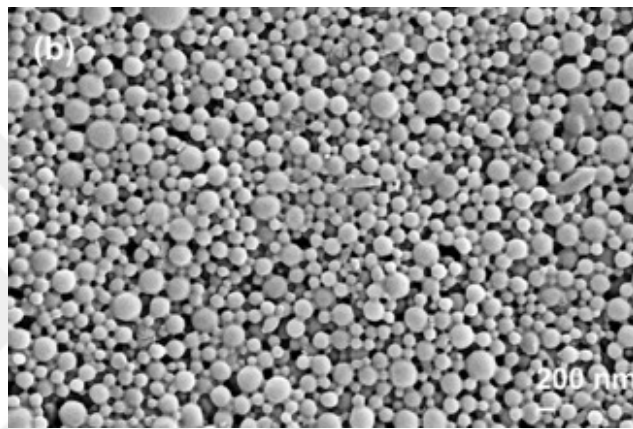
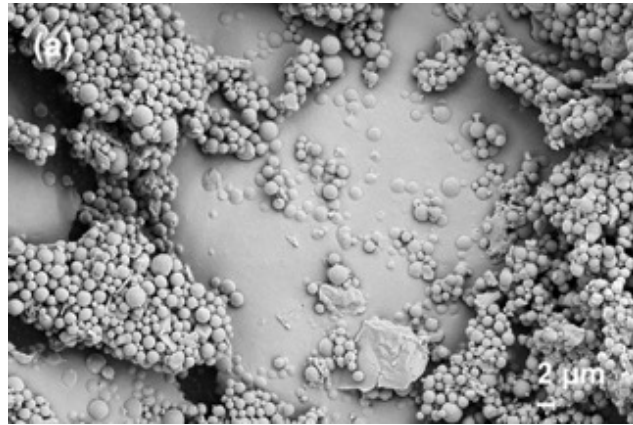


Figure 6.1. (a) Scanning electron microscopy image of 1 μ m and (b) sub-200nm ZnPc loaded PLGA particles, (c) Calculated average size distribution of ZnPc loaded PLGA nanoparticles

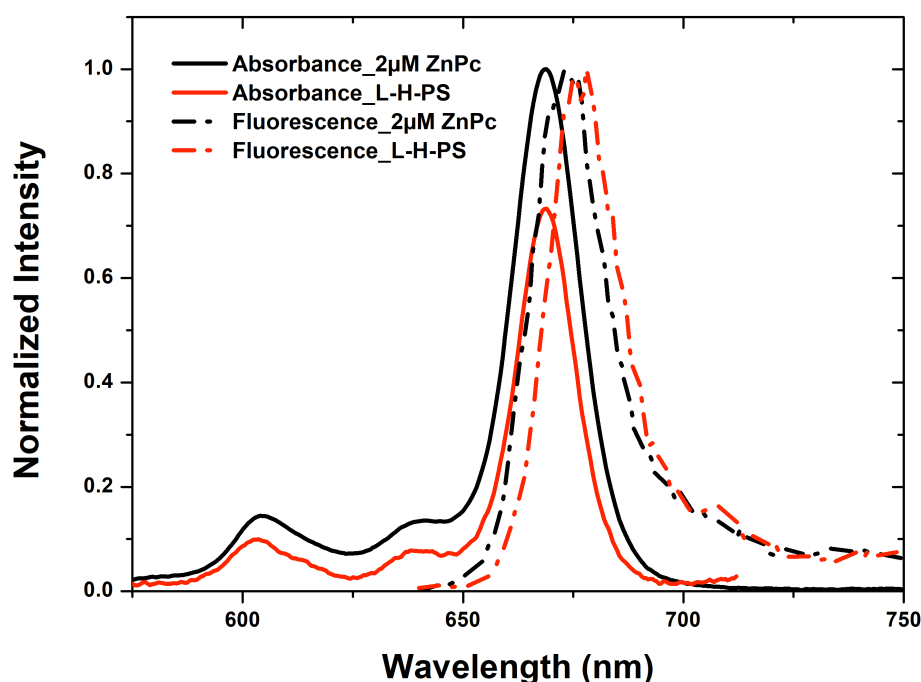


Figure 6.2. Spectroscopic behavior of pure ZnPc and encapsulated ZnPc by copolymer PLGA

6.3.2. Multilayer thin film assembly

Assembly of multilayer thin films is performed onto glass substrates by dip-spin layer-by-layer method. Five bilayers of (bPEI/SPS)₅ polyelectrolyte adhesion pair is coated to enhance the adhesion of the thin film coating containing nanospheres. On top of polyelectrolyte layers, positively charged branched polyethyleneimine (bPEI)/ negatively charged ZnPc encapsulated PLGA nanoparticles (L-H-PS) are homogeneously distributed on the surface. All of the multilayer thin film characterizations are made for the films prepared by the same adhesion pair (bPEI/PSS) and coating material (bPEI/ L-H-PS) with varying number of layers.

Film growth of dip-spin LbL coated glasses is analyzed under UV-VIS spectroscopy. As the number of layers on substrate increases, an increase in absorbance is obtained indicating thin film growth. Homogeneous and uniform coatings are shown in [Figure 6.3a and 6.3b](#) in increasing order of bilayers with the corresponding absorbance curves. During coating, only one-third of the glass slides given in [Figure 6.3a](#) are dip-spin coated. White band on LbL coated glass slides arises due to the slight differences in dipping height of

substrates to the coating material. Homogeneous films are obtained underneath that white band.

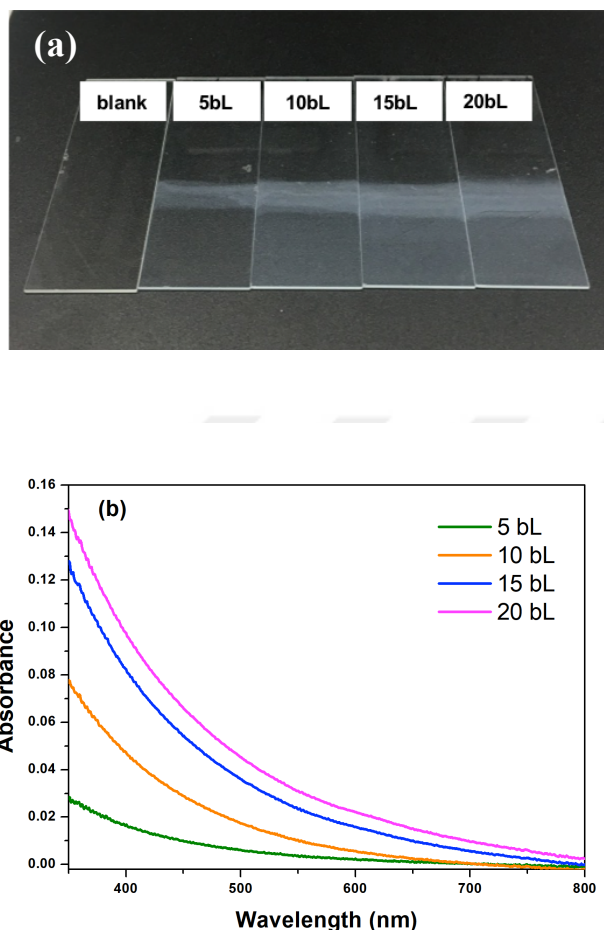


Figure 6.3. (a) Layer-by-layer coated glass substrates by 5,10,15,20 bilayers of bPEI/L-H-PS thin films on adhesion layers, (b) Film growth by absorbance of coatings on glass slides

Surface profiler is used to measure the thicknesses of multilayer thin films and for 5bL bPEI/L-H-PS coating the achieved thickness measured as 63.6 nm. Overall film thickness reaches to 142.8nm for 20bL coating. The results show that multilayer thin film growth is almost linear as given in [Figure 6.4](#) and the thickness per bilayer is determined as 9.3nm. Tailoring drug delivery aspects through thin films is effective when highly customized sets of layers are required especially for building sequential delivery. Hence, the controlled thickness in molecular level is an advantage through this versatile technique as achieved below 10 nm within this study.

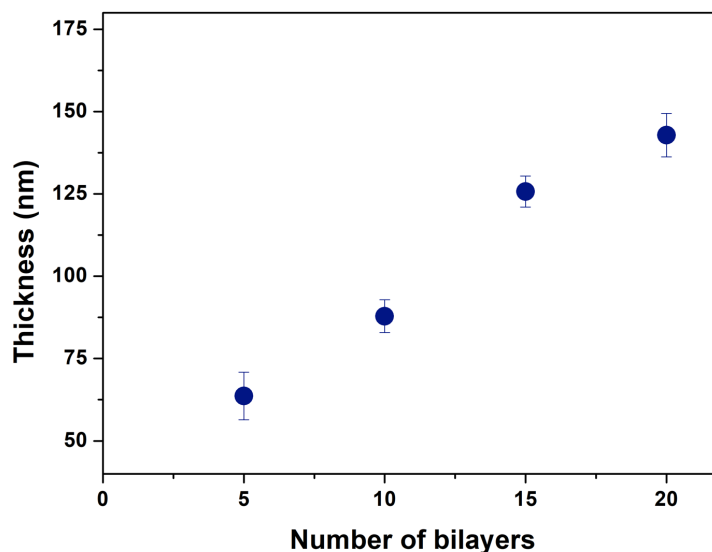


Figure 6.4. Film growth behaviour of bPEI/L-H-PS bilayer architecture

Quartz-crystal microbalance is a mass-sensitive technique. Depending on the adsorption interaction between the coating material and crystal surface, increase in mass affects crystal oscillation frequency. Thus, deposition control is derivable by applying the Sauerbray equation. On a precleaned quartz sensor four bilayers of adhesion pair is coated. After 120 min ZnPc loaded PLGA nanoparticles (L-H-PS) are introduced to the QCM-D system. The sharp decrease in delta-frequency in [Figure 6.5a](#) is due to the assembly of the nanoparticles to the quartz surface. All flat regions after 120 min correspond to positively charged bPEI and wash bath respectively. The flow of nanoparticles is repeated for four times ([Figure 6.5a](#)). During each nanoparticle introduction, identical adsorption tendency is acquired, which is indicating control over nanoparticle amount at each flow among coating layers. Except for change in frequency, thickness and mass variation can be also monitored by QCM-D. Adsorption thickness (nm) with respect to pH change of nanospheres is plotted for 4-bilayers of adhesion pair and 3-bilayers of L-H-PS/bPEI ([Figure 6.5b](#)).

It is proven by QCM-D measurements as shown in [Figure 6.5a and 6.5b](#) that ZnPc loaded nanoparticles are electrostatically assembled to polyelectrolytes coated surface to form a continuous film. Once pH of ZnPc loaded nanospheres (L-H-PS) changes from 2 to 10, adsorption of nanoparticles to the surface is affected. Figure 6.5b represents the

control over film adsorption by changing pH of PLGA nanospheres. Up to eight layers of oppositely charged polyelectrolyte, adhesion coating is consistent on rates in mass change. After eight layers ZnPc loaded capsules are released and introduced to the system. In order to monitor the adsorption tendency at diverse pH conditions an acidic, a basic and neutral pH values are studied. For pH 2 and 5 the adsorption rate in 10 min is 2.01mg/m^2 for nanosphere phase, while raise in pH to 10 decreases the adsorption of nanospheres. In the presence of the basic condition for ZnPc loaded nanospheres molecular interaction between the underneath polyelectrolyte bPEI is poor. In other words, molecular orientation at basic pH leads to the formation of thinner films where the addition of nanospheres causes collapse of bPEI. On the other hand, pH 2 and pH 5 conditions allow efficient molecular orientation which contributes to thicker films by regular buildup. This is especially important when the nanoparticle distribution density is crucial. To avoid toxic effect due to drug overload, nanoparticle density among multilayers should be adjusted which is possible by pH change.

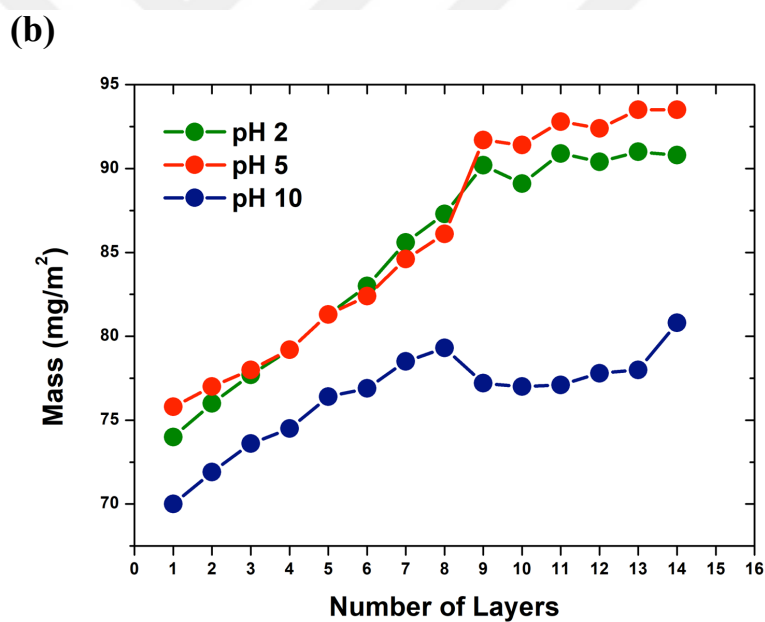
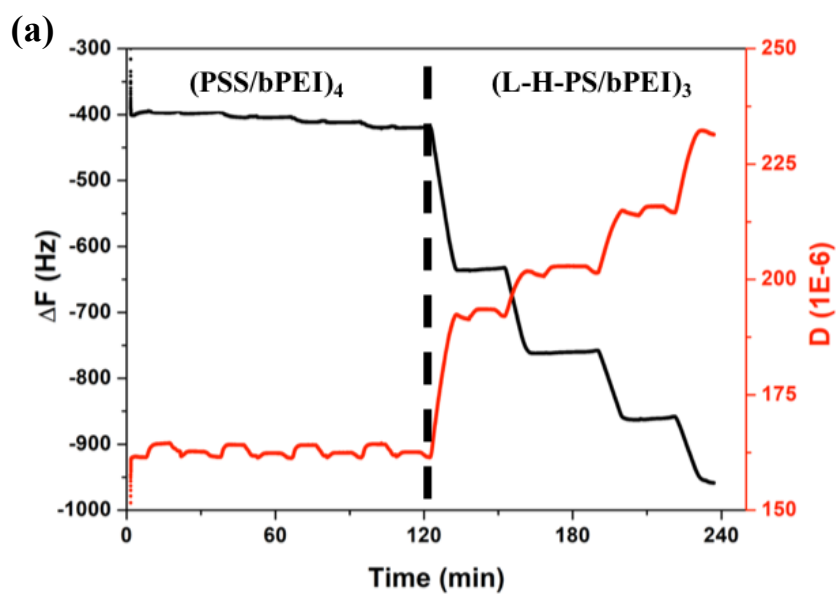


Figure 6.5. (a) Change in frequency and dissipation of thin film coating (PSS/bPEI)₄ up to 120min, (L-H-PS, pH 5.25/bPEI)₃ between 120-240min, (b) Change in adsorbed mass with respect to pH change of nanospheres, 6 layers of (L-H-PS/bPEI) on top of eight layers of (PSS/bPEI)

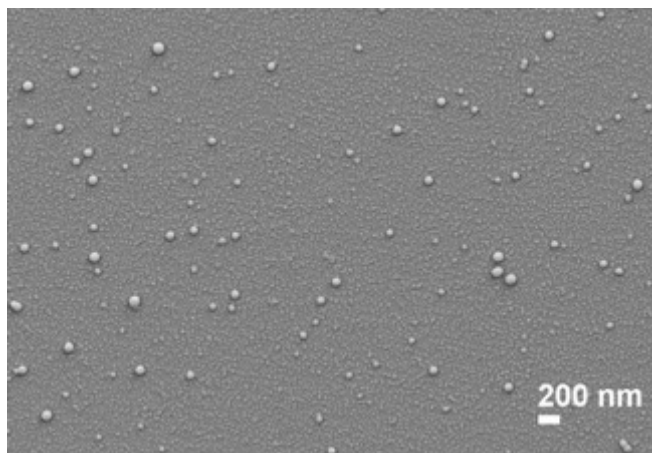


Figure 6.6. Surface morphology of (bPEI/L-H-PS)₅ thin film on adhesion layers

Homogeneous distribution of nanoparticles on the surface is also monitored by secondary electron images of scanning electron microscopy. According to [Figure 6.6](#), approximately 25 ZnPc loaded nanoparticles are present in 1 μm^2 which plays a significant role to avoid quenching during irradiation.

Surface topography and roughness of deposited thin films are investigated by atomic force microscopy ([Figure 6.7](#)). Scan size of both images is 1 $\times$ 1 μm^2 . On the left image the z-scale ranges from -2.8 to 4.1 nm and the corresponding roughness is acquired as 0.88 nm for 5 bilayers of adhesion, where the z-scale range is between -5.9 nm to 8.5 nm with a surface roughness of 1.88 nm for 10 bilayer deposited bPEI/ L-H-PS thin film indicating that a consistent change in the roughness is observed when the deposition of bilayers is increased.

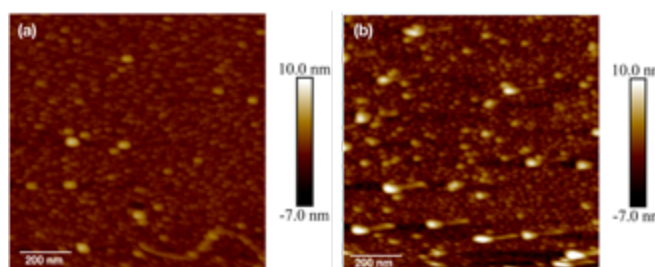


Figure 6.7. Topographical images of (a) adhesion layers (PAH/PSS)₅ and (b) 10-bilayer coated (bPEI/L-H-PS) thin film on adhesion layers

Control over distribution and thickness of ZnPc loaded particles may provide a promising contribution for skin tumor treatments by in-vitro photodynamic therapy in the

near future. It is stated in the literature that controlled surface coatings play a significant role on the effectivity of PDT in terms of toxicity and release¹⁵⁵. Hence, accumulation of photosensitizers will be precluded to avoid toxicity due to overload and activation of photosensitizers will be controlled with increased efficiency as the drug loaded carriers, which are nanospheres in our case, homogeneously distributed. Since PDT is highly recommended for tumors on skin or just under the skin, application of ZnPc loaded PLGA nanoparticles to any biocompatible substrate and irradiation of ZnPc loaded particles by appropriate wavelength can initiate the photodynamic reaction which is essential for singlet oxygen generation. Exposure by light in the visible range destroys tumor cells as a result of generated highly reactive singlet oxygen.

Prepared thin films provide promising results for skin-oriented in-vitro photodynamic therapy applications. There are ongoing clinical trials for various photosensitizer drug molecules. Depending on the photophysical properties, penetration depth and high singlet oxygen yield of the photosensitizer molecules treatment dose is adjusted. An ongoing first-generation photosensitizers Photofrin is one of the clinically approved and applied drug molecules for photodynamic therapy. It has a low molar extinction coefficient (ϵ) of $1170 \text{ M}^{-1}\text{cm}^{-1}$)^{145, 156} and the maximum absorption appears at 630 nm with ~ 1.6 mm penetration depth^{145, 156}. Among second generation photosensitizers Foscan is a promising PDT candidate for clinical trials because of its high molar extinction coefficient of $3.0\text{E}+04 \text{ M}^{-1}\text{cm}^{-1}$ and high singlet oxygen quantum yield^{145, 156}. Hence, the required drug dose of Foscan (0.1 mg/kg) is almost 100 times lower than the required dose of Photofrin (1.5-2 mg/kg) to have a similar response. Phthalocyanines are also counted as strong potent photosensitizers in the literature^{82, 149, 157}. Especially in the presence of a central atom like zinc, silicon or aluminum high singlet oxygen yield and long-lived triplet states are obtained. Molar extinction coefficient for phthalocyanines is quite high ($2.5\text{E}+05 \text{ M}^{-1}\text{cm}^{-1}$)¹⁴⁵. Besides, maximum light absorption for zinc phthalocyanine is higher than the clinically approved Photofrin with 675-701nm¹⁴⁵, indicating deeper light penetration depth.

For an effective dose and corresponding photodynamic response, it is significant to consider both the photophysical properties such as molar extinction coefficient, maximum light absorption- penetration depth and singlet oxygen yield of the photosensitizer molecule. In our study, we have clearly shown that even the encapsulated

zinc phthalocyanine possesses strong photophysical properties in the visible wavelength range, which is known to have a sufficient light penetration depth with more than 2 mm in the visible range in the literature^{144, 158-159} where it is crucial for PDT applications. Therefore, the required dosage of encapsulated ZnPc for a skin-oriented application will be similar or even lower than the sufficient dose of Foscan for a similar response.

6.4. Conclusion

Zinc phthalocyanine loaded PLGA nanoparticles and their multilayer thin films are prepared by co-solvent evaporation and layer-by-layer self-assembly technique. The average size of particles is obtained to be smaller than <100nm that is reported first time in the literature with 86% yield. Among uniform and homogeneous polyelectrolyte multilayers, controllable amount of hydrophobic photosensitizers are distributed by layer-by-layer self-assembly. Maximum absorbance and fluorescence emission intensities are identical with the photophysical properties of the standard ZnPc at 669nm and 674nm respectively. Hence, the co-solvent evaporation method is suitable for encapsulation process without any interference. Synthesized ZnPc loaded PLGA capsules are used for multilayer thin film formation by dip-spin layer-by-layer technique. As the density of ZnPc loaded nanocapsules on surface increases, absorbed amount in the visible wavelength range increases as well. Linear film growth with ~9nm film thickness is achieved per LbL coated bilayer systems. Multilayer film growth is also monitored by the adsorption behavior of nanocapsules on quartz crystals. While the pH of loaded nanocapsules is less than 6, ~2mg/m² adsorption rate is received in 10min. By adjusting the pH of coated nanoparticles, it is possible to control the adsorption amount. ZnPc loaded nanoparticles among thin film multilayers possess strong PDT features for future studies due to deeper light penetration depth and higher maximum light absorption properties than photosensitizer molecules present in the literature.

7. Multilayer Thin Film Deposition of 5,10,15,20-(tera-4-carboxyphenyl) porphyrin and Metallated Phthalocyanine by Layer-by-layer Technique

7.1. Introduction

Thin film engineering of various molecular structures via layer-by-layer deposition method is an ongoing research in materials science. Due to the versatile features of LbL coatings it allows deposition of multiple molecules by a sequential order on distinctive surfaces such as steel, glass and even on drug core. By controlling the order of deposition, distribution of molecular structure among layers and on surface can be adjusted, which enables to reveal desired thin film properties.

Phthalocyanines and porphyrins are well-known macrocyclic compounds which have highly conjugated structures. Their colorful appearance is only one of the favored outcomes of high π -conjugation. In general, due to the hydrophobic character of porphyrins and phthalocyanines application conditions are restricted. However, there is an ongoing research for functionalization of such macrocyclic structures^{46, 160}. Addition of hydrophilic groups such as sulfonic acid, carboxyl or phosphate increases the water compatibility of phthalocyanines and porphyrins. However, there is still a need for a precise control of solubility in aqueous medium by pH. Molecular conformation and ionization degree are strongly dependent on the pH of the medium¹³. Hence, optimum pH conditions are necessary for homogeneous aqueous phthalocyanine and porphyrin solutions. This is especially important for LbL thin film mechanism. Uniform multilayer coatings are only possible with stable aq. solutions.

In the literature, there are some examples for functional assemblies of porphyrins and phthalocyanines¹⁶¹⁻¹⁶⁴. According to Sun et al alternating layers of oppositely charged cobalt porphyrin and phthalocyanine molecules exhibit photovoltaic properties¹⁶⁴. Besides, in 2004 porphyrin-phthalocyanine blends are studied for thin film formation as well¹⁶¹. Although there are numerous examples for sequential molecular deposition of different porphyrin-phthalocyanine pairs and blends, there is a lack in precise control of LbL thin film properties in the presence of polyelectrolytes among layers. In addition, even though the photophysical and photochemical features of these structures in liquid

form are known in detail, any improvement on singlet oxygen generation or lifetime in triplet excited state in solid film form is necessary.

In this study, multilayer thin film engineering of 5, 10, 15, 20-(tetra-4-carboxyphenyl) porphyrin and zinc (II) phthalocyanine tetrasulfonic acid with branched polyethyleneimine is investigated by layer-by-layer method. Obtained coatings are analyzed by UV-Vis spectroscopy and surface profiler for film growth features.

7.2. Experimental Work

7.2.1. Materials

In this study, dip-spin layer-by-layer (LbL) self-assembly technique is applied to coat the substrate surface. As anionic polyelectrolyte poly (sodium 4-styrene sulfonate) (**SPS**, Mw= 70.000) and as cationic polyelectrolyte poly (allylamine hydrochloride) (**PAH**, Mw= 15.000) and branched polyethyleneimine (**bPEI**, MW=25.000) molecules are used. Polyelectrolyte concentrations are set to 10mM, which are based on the molecular weight of the repeating unit. The concentrations of 5, 10, 15, 20-(tetra-4-carboxyphenyl) porphyrin (**TCPP**, Mw=790.77g/mol) and zinc(II) phthalocyanine tetrasulfonic acid (**ZnPcTS**, Mw= 898.17g/mol) molecules are adjusted to 0.1 mM and 0.25 mM. pH values of polyelectrolytes are set to 4.20 for PAH, bPEI and SPS. Aqueous solutions of ZnPcTS and TCPP have pH values of 3.50 and 10.70 respectively.

7.2.2. Layer-by-Layer Film Formation

Layer-by-layer (LbL) deposition is initiated with PAH/SPS polyelectrolyte pair which are assigned as adhesive layers. Substrates are dip-spin coated with PAH solution of 10 mM, pH 4.20 for 10 min at 100 rpm and rinsed with three different distilled water baths for 2/1/1 min respectively. Following that substrates are immersed into 10 mM SPS solution for 10 min and rinsed again with three distilled water baths. This cycle makes a one-bilayer (bL) film and it is repeated five times to obtain five bilayers of PAH/SPS. Once substrates are dip-spin coated with PAH/SPS the same coating procedure is

followed 7 times for bPEI/ TCPP/ bPEI/ ZnPcTS multilayers giving 7 tetralayers (tL) of phthalocyanine and porphyrin containing film. As reference, single component bilayer films of bPEI/ZnPcTS and bPEI/TCPP are prepared as well. Overall film structures and sample codes for selected film systems are shown in [Figure 7.1](#) and [Table 7.1](#) respectively. Both surfaces of all substrates are homogeneously and identically coated.

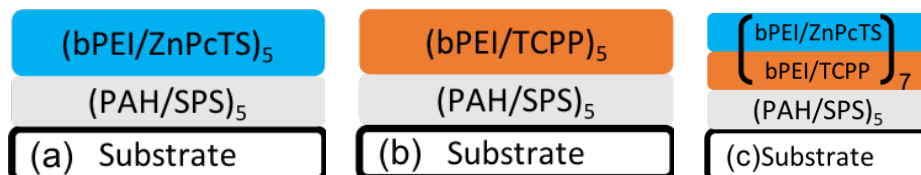


Figure 7.1. (a) bilayer ZnPcTS (b) bilayer TCPP (c) tetralayer film structures

Table 7.1. Studied multilayer thin film systems

	Sample Codes	Concentration (mM)		Coating Information
		Por*	Pc*	
Bilayer	Por_A	0.1	-	(bPEI/Por) ₅
	Por_B	0.25	-	
	Pc_A	-	0.1	(bPEI/Pc) ₅
	Pc_B	-	0.25	
Tetralayer	PorPc_A	0.1	0.1	(bPEI/Por/bPEI/Pc) ₇
	PorPc_B	0.25	0.25	

*Por: 5, 10, 15, 20-(tetra-4-carboxyphenyl)porphyrin; Pc: Zn(II) phthalocyanine tetrasulfonic acid

7.2.3. Characterization

Film growth of prepared coatings is characterized by Cary 5000 UV-VIS-NIR spectroscopy in the visible range. KLA Tencor P6 surface profiler is used for multilayer film growth and thickness analysis. At least seven different points on each substrate surface are measured for an average thickness value.

7.3. Results and Discussion

Multilayer thin film analysis is investigated by the change in absorbance -in the visible wavelength- and in thickness. Porphyrin (Por) and zinc (II) phthalocyanine (Pc) coated thin films are separately examined. Following that tetralayer thin film properties for both TCPP and ZnPcTS containing structures are tested and compared.

According to Figure 7.2 (a) and (b) light absorbance of 0.1 mM and 0.25mM TCPP in the form of (bPEI/ Por)₅ multilayer films are compared with their absorbance behaviors in aqueous phases. As expected, both multilayer films (Por_A and Por_B) and their aq. phases present similar features at 425nm where the characteristic intense Soret band appears. Furthermore, weaker Q-bands of porphyrin are clearly visible above 500 nm for both phases at [Figure 7.2 \(a\) \(b\)](#). All these unique absorbance peaks indicate that TCPP structure is successfully deposited on to substrate surface by LbL self-assembly. Although the concentrations are the same for liquid and solid phases, aqueous solutions exhibit higher absorbance with respect to their multilayer films. During LbL deposition only monolayer molecular assembly is transferred from liquid batch to substrate surface, which is in the order of few nanometers. Hence, the absorbance intensity much lower compared to their aq. phase. When the UV-Vis absorbance of Por coatings is analyzed as in [Figure 7.2 \(b\)](#) 0.1mM concentration shows higher intensity compared to 0.25mM film. Although digital image of Por_B with higher concentration demonstrates darker yellow, its photophysical properties are weaker than Por_A with 0.1mM. This might be due to self-quenching of porphyrin as well.

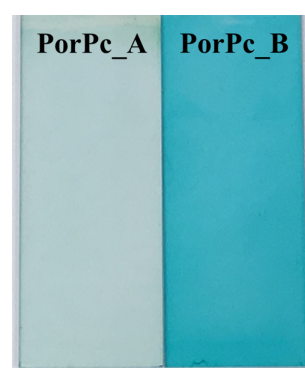
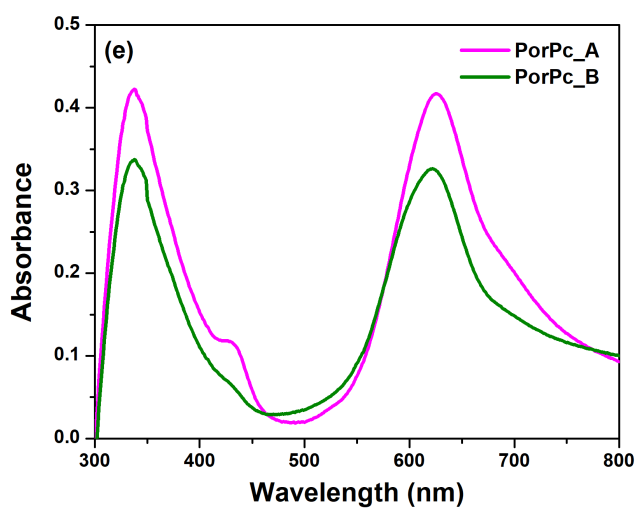
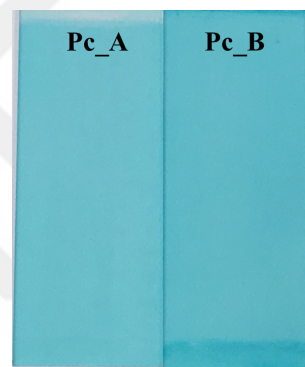
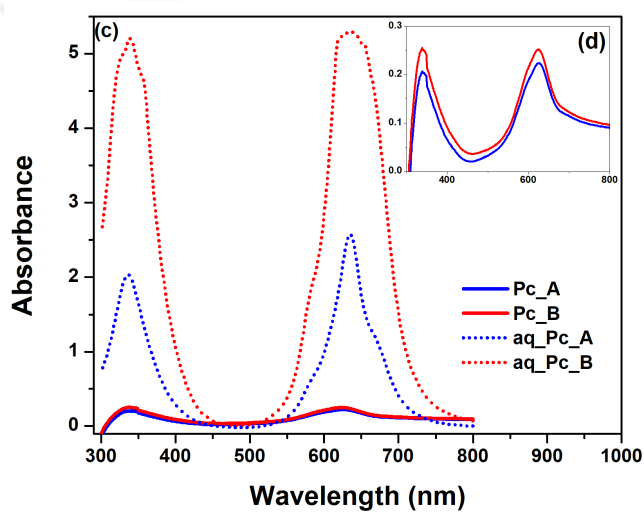
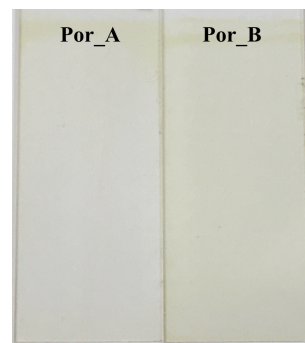
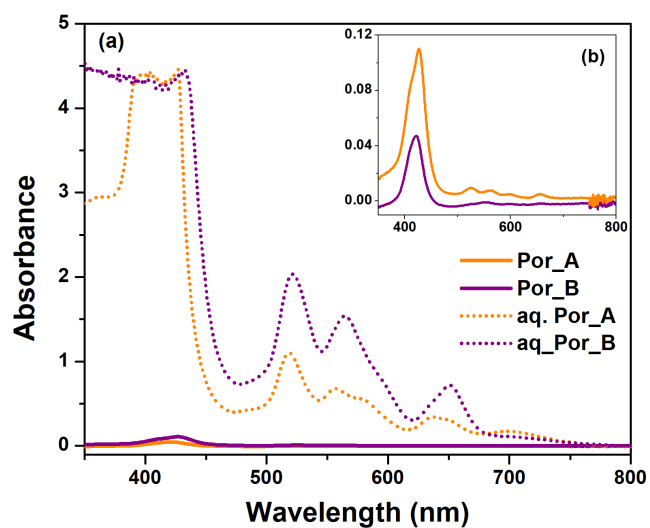


Figure 7.2. Thin film formation and corresponding digital images of (a) Por_A and Por_B, (b) Pc_A and Pc_B (c) PorPc_A and PorPc_B coating systems by UV-VIS spectroscopy

For zinc (II) phthalocyanine introduced coating Pc_A and Pc_B UV-Vis spectra are given in [Figure 7.2 \(c\) and \(d\)](#) with their corresponding film images on glass substrates. Different from Por, phthalocyanines exhibit two strong absorbance peaks between 336-338 nm and 625-635 nm for π to π^* and n to π^* transition respectively. Deposition of ZnPcTS by LbL self-assembly is approved by the obtained absorbance spectra. As expected, higher phthalocyanine concentration presents higher absorbance values even in the form of multilayer coating.

In [Figure 7.2 \(e\)](#) visible spectroscopy of tetralayer films are given which contain both TCPP and ZnPcTS molecules in an alternating order. Near UV-region at 338nm both films PorPc_A and PorPc_B demonstrate the B-band. Shoulder peak around 430 nm can be attributed to the porphyrin molecule. Between 622-626 nanometers another strong absorbance appears which occurs due to the ZnPcTS structure. Although both macrocyclic molecules display their distinguishing transitions on UV-vis spectra in a tetralayer architecture, lower concentration PorPc_A demonstrates higher photophysical properties like the single component film of TCPP, Por_A. UV-visible spectroscopy is an excellent characterization tool to observe aggregation, where photophysical features are significantly affected. In the presence of aggregation self-quenching may occur, which in-activates or reduces photophysical properties of porphyrins/ phthalocyanines.

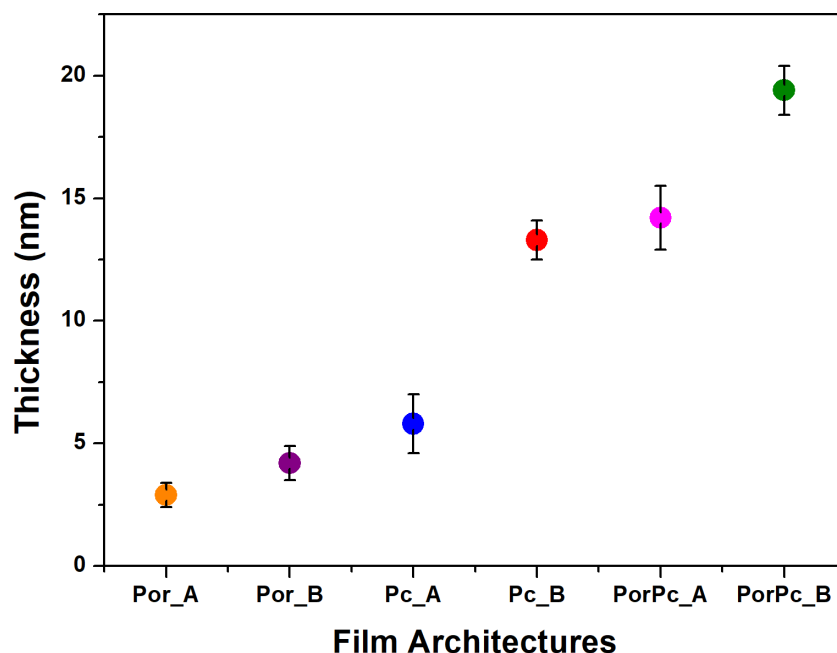


Figure 7.3 Multilayer film thicknesses for studied LbL architectures

Prepared bilayer and tetralayer thin films are measured by surface profiler for thickness analysis. Tetralayer films exhibit thickness from 2.02 to 2.7 nm per tetralayer for PorPc_A and PorPc_B respectively. Among bilayer structures Pc_B with 0.25mM concentration displays greatest thickness with 13.3nm for 5 bilayers. It is known from literature that molecules with lower concentration provide thinner LbL film formation. Hence, in terms of concentration all films with 0.1mM show thinner coatings compared to multilayers prepared with 0.25mM.

7.4. Conclusion

In this chapter, influence of concentration for TCPP and ZnPcTS molecules on photophysical properties of multilayer coatings fabricated via layer-by-layer self-assembly is explored. It is determined that higher concentration of macrocyclic compounds may lead aggregation formation which decreases the absorbance in visible region by self-quenching. Therefore, critical concentration of porphyrin/ phthalocyanine molecule needs to be determined for future potential surface-based applications.

8. Summary and Conclusions

The presented study describes novel multilayer thin film coatings by layer-by-layer (LbL) approach for potential future applications. Control over certain parameters in LbL technology such as pH, concentration of coating materials and surface distribution simplify nano-scale film management. This doctoral thesis is divided into four main chapters, where the impact of pH and concentration for nickel(II)phthalocyanine tetrasulfonic acid tetrasodium salt on LbL coated films is analyzed in Chapter 4. Subsequently, in Chapter 5 corrosion resistance of NiPcTS and CuPcTS embedded multilayer LbL coatings is investigated. In Chapter 6, control over thin film properties are examined for encapsulated zinc phthalocyanine for potential photodynamic therapy purposes. Finally, different thin film architectures for 5,10,15,20-(tetra-4-carboxyphenyl) porphyrin and zinc(II)phthalocyanine tetrasulfonic acid containing multilayers are explored and compared with 0.1 mM and 0.25 mM concentrations by layer-by-layer self-assembly in the 7th Chapter.

In chapter 4, 0.1 mM and 0.25mM concentrations of nickel(II)phthalocyanine tetrasulfonic acid tetrasodium salt in a wide pH range is examined in terms of thin film properties by layer-by-layer self-assembly. Maximum absorbance of NiPcTS at 614 nm is sustained while transferring phthalocyanine in aqueous solution to solid substrate surface. As the number of bilayers is increasing from 2 to 10, intensity of Q-band on absorbance spectroscopy increases as well. 0.25 mM NiPcTS concentration exposes thicker and higher absorbance intensities compared to (bPEI/NiPcTS)_n film prepared with 0.1 mM NiPcTS concentration. Exponential film growth can be explained by layer interpenetration or additional molecular interactions beside electrostatic interactions. Among a set of pH values acidic pH at 2.5 gives the highest thickness and absorbance, which is clearly visible from the darker blue appearance of coating. 80.9 nm and 98.3 nm are the measured highest thickness values of 10 bilayers of (bPEI/NiPcTS) coating prepared with 0.1 mM and 0.25 mM NiPcTS concentration at pH 2.5 respectively.

In the 5th chapter, in addition to NiPcTS, CuPcTS is also embedded among oppositely charged polyelectrolyte layers separately in bilayer and tetralayer architectures by LbL process. Obtained homogeneous and uniform films are examined for corrosion resistance in the presence of dilute Harrison's solution. Both phthalocyanines maintain their

photophysical properties once they are transferred to solid surfaces. While bilayer films A1 and A2 show linear growth, tetralayer coating structures T1, T2, P1 and P2 provide exponential growth behavior, which can be attributed to increases polyelectrolyte concentration in tetralayer system. 7.3 nm/bilayer and 6.9 nm/ tetralayer are the highest thickness values measured for A1 and P1 structures respectively. According to the electrochemical analysis, electroactivity of NiPcTS and CuPcTS containing multilayers are justified by cyclic voltammetry. In the presence of nickel-centered phthalocyanine at A1 architecture, 10-fold decrease in corrosion rate is obtained. However, as well as concentration of electroactive material among layers, their distribution is also highly significant. Tetralayer composition T1 and P1 possess higher capacitive features.

In chapter 6, ZnPc loaded PLGA capsules with less than 100 nm size are developed by oil-in water (o/w) emulsion-solvent evaporation method with 86% yield. It is confirmed by fluorescence spectroscopy that encapsulation does not interfere with zinc phthalocyanine's photophysical properties. ZnPc loaded capsules paired with branched polyethyleneimine are homogeneously coated on glass substrates by dip-spin LbL method. 142.8 nm thickness is measured for 20 bilayers of coating by surface profiler. Sequential deposition is also verified by quartz-crystal measurement and 2.01 mg/m² adsorption rate is obtained in 10 minutes for nanospheres at pH 2 and 5. Surface roughness for 10 bilayer film is acquired as 1.88 nm. Control over adsorption amount of photosensitizer containing capsules can demonstrate promising photodynamic features for future skin-oriented applications due to high light absorbance and deep light penetration properties of zinc(II) phthalocyanine.

In the final chapter 7, alternating bilayer and tetralayer coatings of 5,10,15,20-(tetra-4-carboxyphenyl) porphyrin and zinc(II)phthalocyanine tetrasulfonic acid paired with branched polyethyleneimine are studied by dip-spin LbL technique on glass substrates. Different from investigated phthalocyanine compounds, porphyrin embedded bilayer film shows higher absorbance for lower 0.1 mM concentration compared to its 0.25 mM concentration sample. Similarly, tetralayer coating structure with both 0.1 mM porphyrin and 0.1 mM phthalocyanine contribution exhibits higher absorbance, which is associated with self-quenching of porphyrin. In terms of thickness, all multilayer films prepared with lower phthalocyanine or porphyrin concentration (0.1 mM) reveal thinner coatings.

Overall in this thesis, various structural combinations of oppositely charged polyelectrolytes and phthalocyanine-based compounds are studied by layer-by-layer deposition mechanism. By adjusting pH, concentration, surface distribution and coating design thin film properties are controlled, which become crucial when the obtained results are transferred to application.



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Research Outcomes

Publications

Belce, Yonca; Cebeci, Ç. Fevzi. (April, 2019). “*Multilayer Thin Films of Zinc (II) Phthalocyanine loaded Poly(D,L-lactide-co-glycolide) Nanocapsules by Layer-by-Layer Self Assembly*”. Journal of Materials Research Express (available online: <https://dx.doi.org/10.1088/2053-1591/ab1278>)

Belce, Yonca; Cebeci, Ç. Fevzi. (Jan, 2019). “*Investigation of pH and concentration influence on layer-by-layer self-assembly for nickel(II)phthalocyanine-tetrasulfonic acid tetrasodium salt coatings*”. Journal of Porphyrins and Phthalocyanines, special issue for Women in Porphyrin Science. (available online: <https://doi.org/10.1142/S1088424619500032>)

Belce, Yonca; Cebeci, Ç. Fevzi. “*Phthalocyanine based multilayer thin film coatings for corrosion protection*”. Manuscript is ready for submission.

Belce, Yonca; Chen, Kepeng; Zhao, Jianzhang; Cebeci, Ç. Fevzi, Fabienne Dumoulin. “*Multilayer Thin Film Deposition of 5, 10, 15, 20-(tetra-4-carboxyphenyl) porphyrin and zinc (II) phthalocyanine tetrasulfonic acid by Layer-by-Layer Technique*”. Manuscript is ready for submission.

Conference Presentations

Oral:

Belce, Yonca; Cebeci, Ç. Fevzi. (2018, August). *Thin Film Engineering of Hydrophobic Phthalocyanine containing Nanospheres by Layer-by-Layer (LbL) Method*. 256th ACS (American Chemical Society) National Meeting & Exhibition, Boston, USA.

Belce, Yonca (2018, May). *Controllable capsules among layers on bandages*. International 3MT (3 Minute Thesis) Competition, Sabancı University

Belce, Yonca; Cebeci, Ç. Fevzi. (2018, May). *Multilayer Thin Films of Hydrophobic Phthalocyanine loaded Nanoparticles by Layer-by-Layer (LbL) Technique*. 3rd GTU (Gebze Technical University) Photodynamic Day, Gebze, Turkey.

Belce, Yonca; Cebeci, Ç. Fevzi. (2017, Nov). *Phthalocyanine-based Thin Film Coatings by Layer-by-Layer Assembly for In-Vitro Photodynamic Therapy*. 2017 MRS Fall Meeting & Exhibition, Boston, USA.

Poster:

Belce, Yonca; Cebeci, Ç. Fevzi. (2017, Sept). *Phthalocyanine-based Thin Film Coatings by Layer-by-Layer Method for In-Vitro Photodynamic Therapy*. 17th EPS (European Society for Photobiology) Congress, Pisa, Italy.

Belce, Yonca; Cebeci, Ç. Fevzi. (2017, May). *Thin Films of Phthalocyanines Fabricated by Layer-by-Layer Method for In-Vitro Photodynamic Therapy*. 2nd GTU (Gebze Technical University) Photodynamic Day, Gebze, Turkey. **Best Poster Award**

Yakut, Yonca; Cebeci, Ç. Fevzi. (2015, May). *Phthalocyanine Based Advanced Corrosion Resistant Coatings by Layer-by-Layer Technique*. 227th ECS (The Electrochemistry Society), Chicago, USA

9. Appendix

9.1. Appendix A

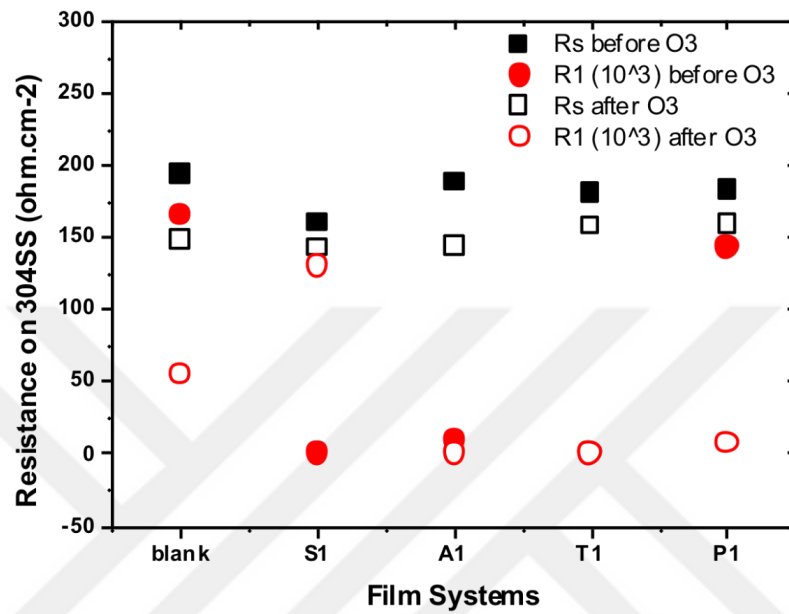


Figure 9.1. Coating resistance before and after ozone treatment

9.2. Appendix B

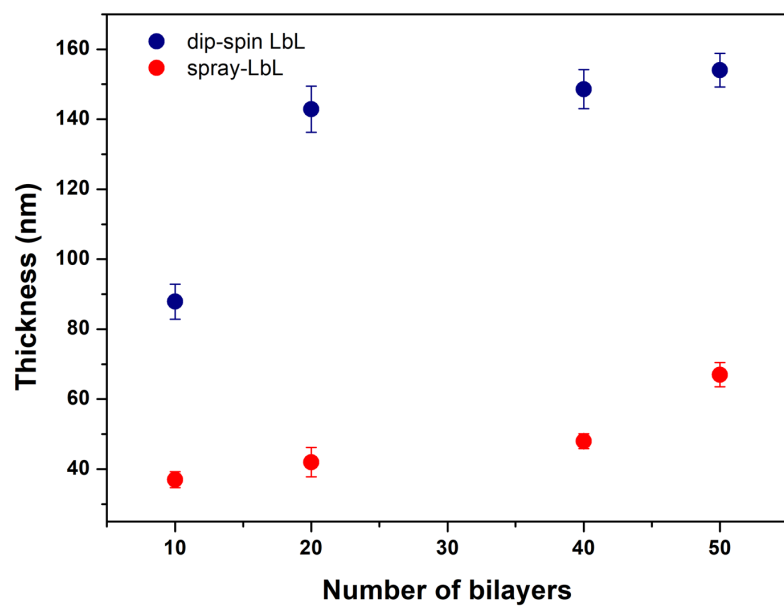
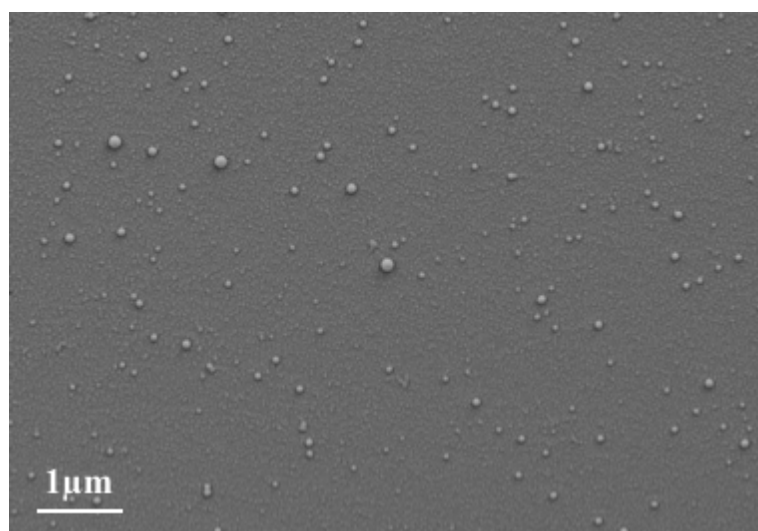
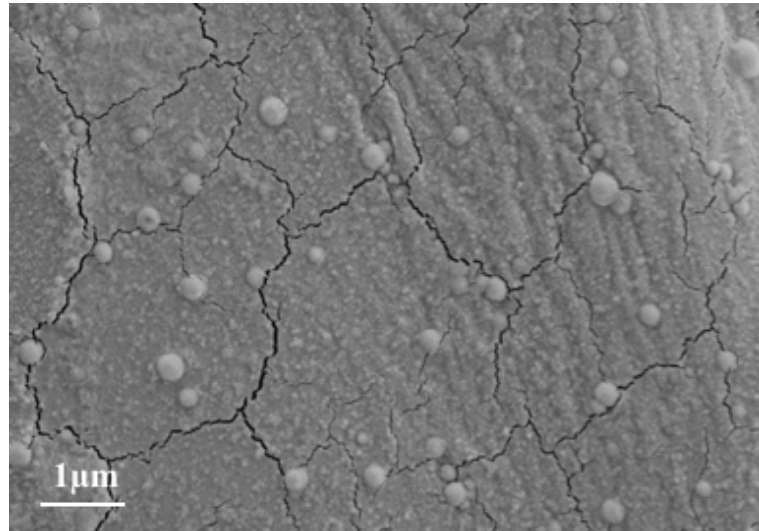


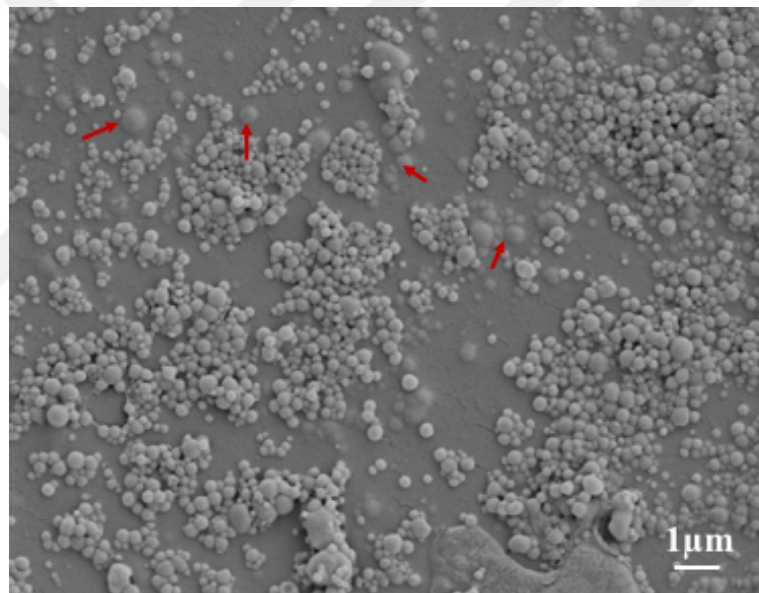
Figure 9.2. Thickness comparison for dip-spin vs spray LbL coatings of nanospheres on glass substrates



(A)



(B)



(C)

Figure 9.3. SEM images of (A) 5bL of film on si-wafer, (B) 5 bL film on sponge, (C) 50 bL film on sponge

9.3. Appendix C

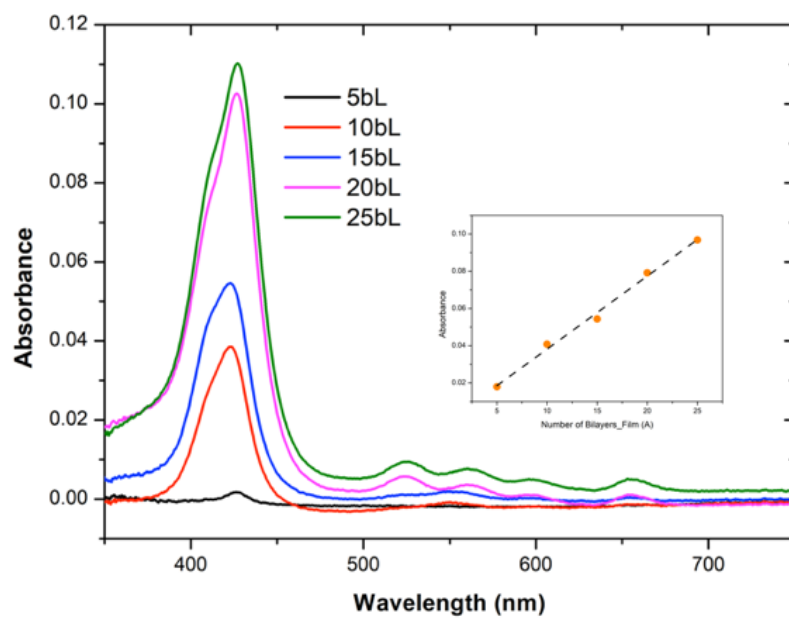


Figure 9.4. Multilayer film growth of $(10 \text{ mM bPEI}/0.25\text{mM TCPP})_n$ by UV-Vis spectroscopy

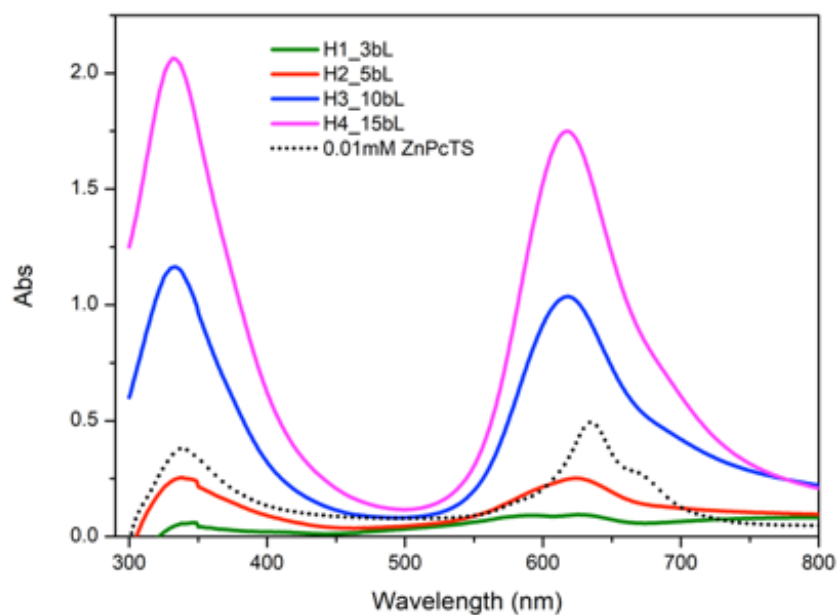


Figure 9.5. Multilayer film growth of $(10 \text{ mM bPEI}/0.25\text{mM ZnPcTS})_n$ by UV-Vis spectroscopy