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Faculty of Science

Effect of pH and detergents on the activity of enzymes immobilized by the layer-by-layer approach

Bachelor thesis

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Annotation

In this thesis, the immobilization of Horseradish peroxidase in polyelectrolyte multilayers prepared by the layer-by-layer approach is studied. The layers have been prepared using polystyrene sulfonate (PSS) and polyallylamine hydrochloride (PAH) dissolved in Tris-buffered saline at different pH levels and in the presence of sodium dodecyl sulfate (SDS). The activity of the immobilized enzyme was analyzed, and its presence and identity were confirmed by immunoblotting.

Declaration

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

České Budějovice, 14.12.2023

Student's signature

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1. Introduction

1.1 Polyelectrolytes and Applications

Polyelectrolytes are macromolecular compounds with repeating units, that dissociate into charged molecules in solution. They form polymeric chains bearing either a positive or negative net charge. The charge on the polyelectrolyte and the consequential electrostatic interactions influence the pH of the solution, the viscosity, and the solubility of the polyelectrolyte, among other changes in physical properties. Due to this variability in physical properties, polyelectrolytes have numerous applications in pharmaceutical and biomedical applications but also in food, cosmetics, and in water treatment [1].

[Polyelectrolyte + drug] complexes have been investigated as injection formulations for site-specific drug delivery. Polymer coatings have been investigated as a way for drug release in response to certain physiological stimuli, e.g., pH-dependent swelling of polyelectrolytes [1].

1.2 Layer-by-layer method

The first use of Layer-by-Layer (LbL) assembly of a multilayer coating is attributed to J. J. Kirkland in 1965. Glass beads used as gas chromatography supports were coated with thin layers of diatomaceous earth or silica to increase the efficiency of the chromatography [2]. In the same year, R. K. Iler of DuPont described the general technique and possible uses, using silica particles and alumina fibrils [3]. In the early 90´s, Decher and his colleagues were among the first to produce multilayers using polyelectrolytes [4] [5].

It is only in the last two decades, that this topic has received attention, and it is now a heavily studied topic, as it has many potential uses in different areas of research such as optics, energy, separations, biomedicine, etc.

Using the LbL method, multilayer coating of substrates using various materials can be produced, by depositing alternating layers of oppositely charged compounds on a surface. The absorption leads to single monomolecular layers that are ~ 1 nm thick [6]. The electrostatic forces of the layer materials (e.g., polyelectrolytes, biopolymers, clays, or metal) allow for facile self-assembly. In combination with the ionic strength, the coating time, the pH of the solution, different additives, as well as the preparation method, they influence the physical properties of the multilayers [7].

The substrate can be coated in multiple ways, ranging from simple dipping (Fig. 1), or spraying the starting material to magnetic assembly, microfluidics, or electrodeposition. All methods achieve an alternating pattern of positively and negatively charged compounds forming a layer in the range of nanometers on the surface of the substrate [8]. The possibility to easily automate the production of layers allows for large-scale applications and low production costs.

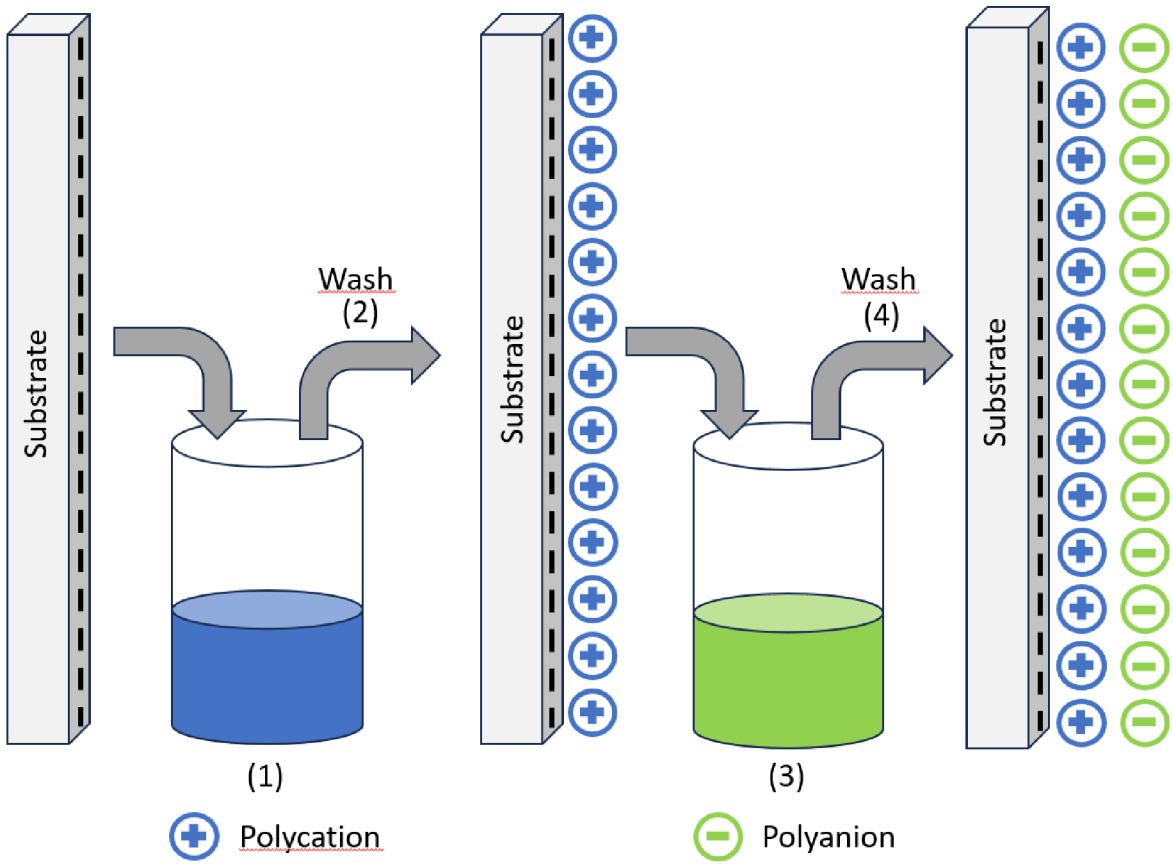

Figure 1: A schematic of the deposition of oppositely charged materials using the LbL method. First, a layer of polycations is applied (1), followed by washing (2), and the application of polyanions (3), and another wash (4). These steps are repeated until the desired number of layers is reached.

A wide range of applications is available for the LbL technique. In 1996, it was used to assemble LEDs, where the choice of polymer and the thickness of the constructed layer influence the brightness and the turn-on voltage [9]. In thin-film coatings of glass slides, multilayers have been proven to have antireflective, antifogging, and self-cleaning properties [10].

Using polyelectrolytes, the surfaces of drug preparations can be coated to enhance the dissolution rate and ensure sustained drug delivery. Due to the low pH of the stomach, many drug preparations use high dosages to account for the destruction of active ingredients by gastric acid. Using mesoporous silica nanoparticles coated by polyelectrolytes, the

preparations can be coated, and only small amounts of drugs are released at gastric pH. Consequently, the release takes place at a later stage of digestion, where the pH is higher, and the absorption of the drug is enhanced. The dosage of drugs can therefore be reduced to minimize side effects, for example in chemotherapy drugs [11].

1.3 Horseradish peroxidase (HRP)

HRP is a relatively cheap plant enzyme, that is stable at a pH range from 5 to 9. In combination with H_2O_2 as an oxidant, HRP is a catalyzer for the oxidation of various substrates forming colored products. 3,3',5,5'-Tetramethylbenzidine (TMB) can be used as a substrate for the determination of HRP activity, when a stable blue product is formed, that can easily be measured by UV-VIS spectroscopy at around 380 nm and 650 nm. After the addition of a strong acid, a yellow product is formed that is measurable at around 450 nm. The low toxicity and the stability of the products ensure a simple procedure [12].

HRP is a heme-group containing enzyme. There are more than 15 isoenzymes that exist in the plant, of which C1A is the most studied due to its abundance. This isoenzyme consists of 308 amino acids and has a molecular weight of 34 kDa. When expressed in a plant, the enzyme has a carbohydrate content of roughly 20 % resulting in an increased mass of 44 kDa [13].

2. Aim of the work

Enzymes play a significant role in medicinal, industrial, and research applications. In their free form, they are vulnerable to environmental challenges, which influence their activity or can denature the protein structure. Numerous immobilization techniques have been developed, to improve the usability of enzymes in large-scale applications. One of these techniques is immobilization on a polymer/polyelectrolyte support created by the layer-by-layer technique.

As the membranes created by the layer-by-layer approach can enclose bioactive molecules, their activity can be influenced by the environmental conditions while constructing the multilayer. Thus, the aims of this work were:

- Immobilization of Horseradish Peroxidase (HRP) in polyelectrolyte multilayers,
- Determination of the dependence of HRP activity on the pH in the buffer during multilayer preparation,
- Determination of the dependence of HRP activity on the presence of a detergent, Sodium dodecyl sulfate, in the buffer during multilayer preparation.

3. Material and Methods

3.1 Constructing the Layers

In order to perform the layer-by-layer technique, layers of polyelectrolytes were deposited. The anchoring layer consisted of Polyethyleneimine (PEI, Fig. 2) (Mw = 60 kDa, Mw(monomer) = 43.04 kDa) followed by alternating layers of Polystyrene sulfonate (PSS, Fig. 3) (Mw = 70 kDa, Mw(monomer) = 206.19 kDa) and Polyallylamine hydrochloride (PAH, Fig. 4) (Mw = 17.5 kDa, Mw(monomer) = 92.55 kDa). Horseradish peroxidase (HRP) was used as an enzyme to be immobilized between the layers and consequently detected.

The polyelectrolytes were dissolved in Tris-buffered saline (TBS, Table 1) to produce a concentration of 100 mM (Table 2), and the solutions were filtered through 0.22 µm syringe filters.

The enzyme was dissolved in TBS buffer as well at a concentration of 0.4 µg/ml (Table 3).

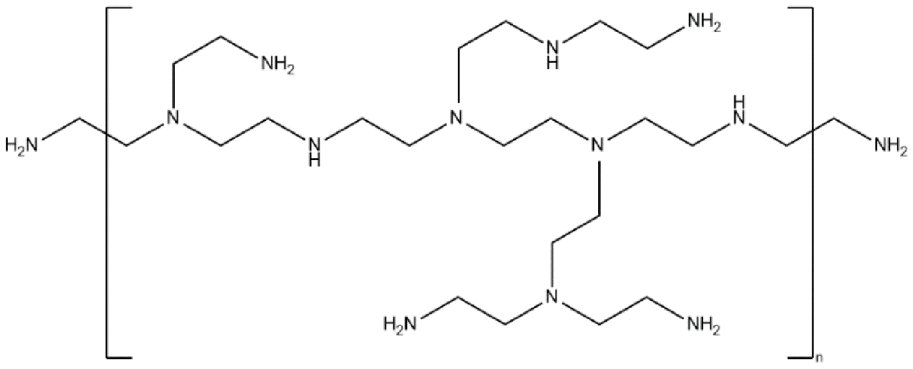

Figure 2: Structure of Polyethyleneimine (PEI), produced with ChemDraw 21.0.0

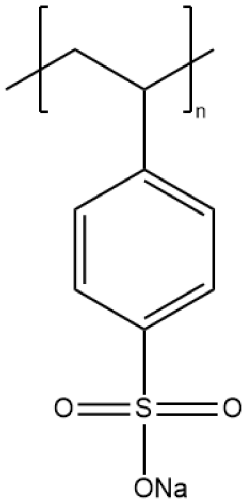

Figure 3: Polystyrene sulfonate (PSS), produced with ChemDraw 21.0.0

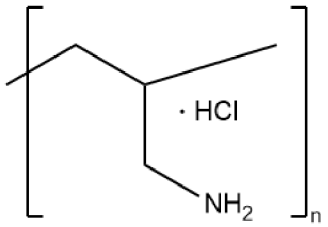

Figure 4: Structure of Polyallylamine hydrochloride (PAH), produced with ChemDraw 21.0.0.

Table 1: Composition of Tris-buffered saline (TBS) used to dissolve the polyelectrolytes and HRP.

Tris-buffered saline (TBS)	**Tris base**	**NaCl**	**Deionized H_2O**	**Notes**
Concentration	20 mM	500 mM	-	
Amount	2.42 g	29.24 g	~ 0.8 l	pH = 7.5 (adjusted using HCl)

Table 2: Composition of the polyelectrolyte solutions used to construct the layer.

	Concentration	**Mass**	**Volume of TBS**
PEI	100 mM	0.02 g	5 ml
PSS	100 mM	0.1g	5 ml
PAH	100 mM	0.04 g	5 ml

Table 3: Preparation of the HRP solution.

	Stock concentration (in TBS)	**Final Concentration**	**Composition**
HRP	10 mg/ml	0.4 µg/ml	0.2 µl stock + TBS to 5 ml (diluted in steps due to small amount)

The layers were prepared in triplicates in 24-well plates, using microscope cover glasses (Ø=15mm) as a base in each well, and coating it with PEI. The coating was performed by pipetting 250 µl of polyelectrolyte into each well and incubating for 15 minutes at room temperature. For the enzyme, 250 µl were used as well, but it was incubated for 30 minutes. Between each layer, the wells were washed with TBS for one minute. The following scheme was used for all experiments.

Table 4: Scheme for layer construction

V1	V2	V3	V4	V5	V6	V7	V8
glass	glass	glass	glass	glass	glass	glass	glass
PEI	PEI	PEI	PEI	PEI	PEI	PEI	PEI
PSS	PSS	PSS	PSS	PSS	PSS	PSS	PSS
PAH	PAH	PAH	PAH	PAH	PAH	PAH	PAH
PSS	PSS	PSS	PSS	PSS	PSS	PSS	PSS
PAH	PAH	PAH	PAH	PAH	PAH	PAH	PAH
	Enzyme	Enzyme	Enzyme	PSS	PSS	PSS	PSS
		PAH	PAH	PAH	PAH	PAH	PAH
		PSS	PSS	PSS	PSS	PSS	PSS
			PAH	PAH	PAH	PAH	PAH
			PSS		Enzyme	Enzyme	Enzyme
						PAH	PAH
						PSS	PSS
							PAH
							PSS

Once the layers were built up, a measurement of the activity of the enzyme was performed by adding 100 µl of 3,3´,5,5´-Tetramethylbenzidine (TMB) as a substrate into each well to form a blue product. After transferring the solution to a 96-well plate, the intensity of the color was measured with a Microplate reader at 370 nm and 655 nm. In the first experiments, the solution was then additionally acidified with 50 µl of 0.5 M sulfuric acid to form a yellow product and measured at 450 nm.

3.2 Preparing the samples for SDS-PAGE

The presence of the enzyme in the multilayers was confirmed through SDS-PAGE and immunoblotting. As the presence of polyelectrolytes interfered with the electrophoresis, the enzyme had to be extracted using methanol chloroform precipitation. After washing the wells thoroughly with TBS, 50 µl of 1x non-reducing loading dye diluted from 5x non-reducing loading dye (0.313 M Tris, pH 6.8, 10 % w/v SDS, 5 % glycerol, 0.05 % bromophenol blue, pH 6.8) for SDS-PAGE were added, and the plate was placed on a shaker at 50 °C for

15 minutes. The solution was then transferred into microtubes and 200 µl methanol were added. After vortexing, 50 µl chloroform were added and the tubes were again vortexed. Next, 150 µl deionized H_2O were added and after vortexing a third time, the tubes were placed in a centrifuge and spun for two minutes at 14 000 x g. Two layers had formed, and the top aqueous layers were pipetted off, as the enzyme (protein) was present between the layers. Again, 200 µl of methanol were added and the tubes were vortexed, then spun for 3 minutes at 14 000 x g. The enzyme had formed a pellet and as much liquid as possible was pipetted from the tube. To dry the pellet further, they were placed in a SpeedVac, careful not to dry it too long which would make it hard to resolubilize. The pellet was then redissolved in 50 µl of 1x non-reducing loading dye.

3.3 Various conditions

To analyze how different pH values and the presence of detergents influence the activity of the enzyme fixed in the layers, the following conditions of buffers were used for the construction of the layers:

- pH = 5
- pH = 7.5 (standard)
- pH = 9
- containing 0.1 w% SDS, pH 7.5
- containing 1 w% SDS, pH 7.5

The activity of HRP was then analyzed using TMB as a substrate and measuring the absorption as performed in Chapter 3.1.

3.4 SDS-PAGE

The samples and Protein Marker VI (10 – 245) (prestained PanReac AppliChem protein marker) were loaded onto a 12 % polyamide gel with 5 % stacking gel (Table 5), both containing trichloro ethanol (TCE), and electrophoresis was performed in 1x running buffer (0.25 M Tris, 1.92 M Glycine, 1 % SDS) at 100 V for approximately 90 minutes until the blue loading buffer reached the end of the gel. The gel was then analyzed under UV light in a gel documentation system, where the TCE bound to proteins is visible (stain-free detection).

Table 5: Components of the gels used in the SDS-PAGE

Components	12 % separation gel	5 % stacking gel
30 % Acrylamide solution	2 ml	165 µl
4x separation buffer	1.25 ml	
4x stacking buffer		250 µl
dd H_2O	1.7 ml	575 µl
10 % Ammonium persulphate	50 µl	10 µl
TEMED	2 µl	1 µl
TCE	25 µl	5 µl

Table 6: Composition of the Separation buffer and the Stacking buffer used in the gel preparation for the SDS-PAGE

4x Separation buffer	Concentration
Tris base	1.5 M
SDS	0.4 %
HCl	to pH 8.8
4x Stacking buffer	**Concentration**
Tris base	0.5 M
SDS	0.4 %
HCl	to pH 6.8

3.5 Immunoblotting/western blot

To further visualize the enzyme and confirm the identity of the signals present in the SDS-PAGE, immunoblotting was performed. A piece of nitrocellulose membrane as well as four pieces of filter paper were cut and placed in blotting buffer (0.25 M Tris-HCl, 1.92 M Glycine). The gel was washed with distilled water and placed in blotting buffer as well. Then the electroblotting apparatus was assembled (Fig. 5) and electroblotting was performed at 15 V for 60 minutes.

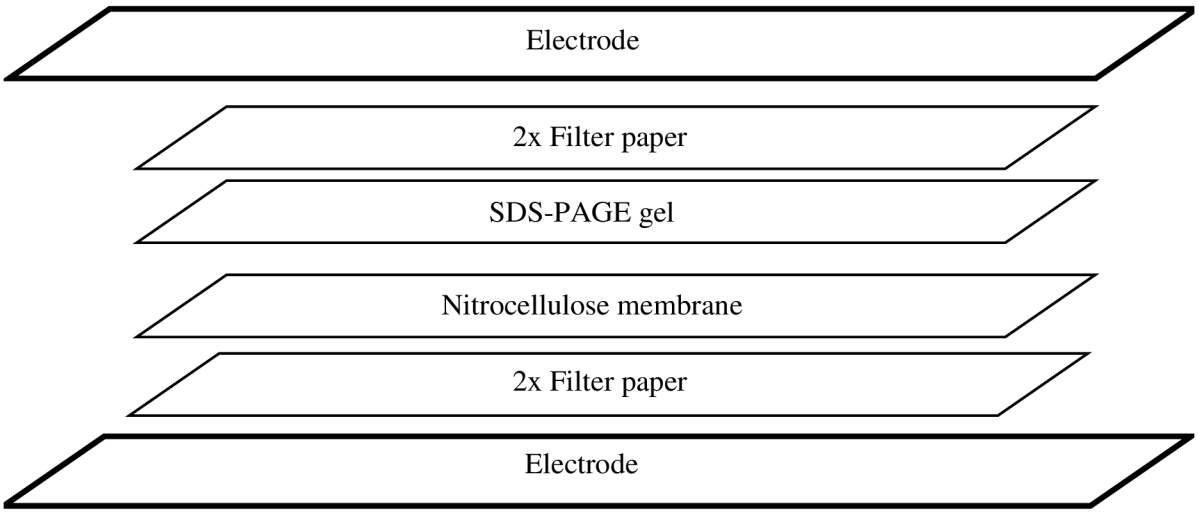

Figure 5: Arrangement of the components of an electroblotting sandwich

The membrane was then placed in a blocking solution (5 % dried milk in 1x PBS (80 g NaCl, 2 g KCl, 14.4 g NaH_2PO_4, 2.4 g KH_2PO_4, ddH_2O to 1 L)) and stored in the fridge overnight. The next day, the membrane was washed three times in PBS and shaken in a blocking solution containing the primary antibody (Anti-HRP produced in rabbit) at a 1:2000 dilution for one hour. Then it was washed again three times in PBS-T (0.05 % Tween-20 in 1x PBS) and incubated with the secondary antibody (Anti-Rabbit produced in goat conjugated with alkaline phosphatase (AP)) in a blocking solution at a dilution of 1:1000 for another hour. Finally, the membrane was washed two times in 1x PBS-T and one time in PBS before placing it in AP staining solution (AP developing buffer 10 ml, 66 µl NBT in 70 % DMSO, 33 µl BCIP in 100 % DMSO) and developing until bands were visible on a light background.

4. Results

4.1 Layer construction and measurement of HRP activity

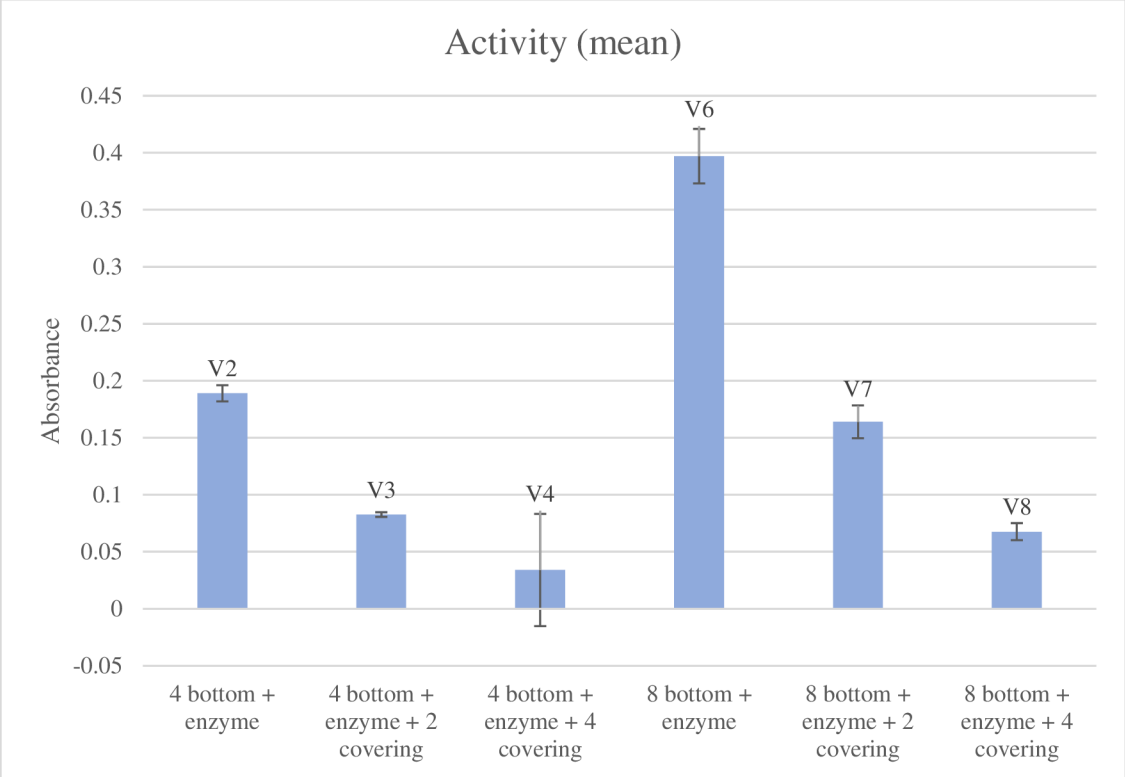

Figure 6: Activity of HRP after addition of TMB; measured using a spectrophotometer at 370 nm; All values have been normalized by subtracting the value of the negative controls (V1 and V5).

The mean absorption has been adjusted in comparison to the negative control (without enzyme). Therefore, only the additional activity of the added HRP is shown in all of these graphs. Adding four additional underlying layers increased the activity. The more layers of polyelectrolyte are deposited above the enzyme, the lower the activity, as the enzyme is more strongly shielded.

4.2 Varied conditions

4.2.1 pH = 5

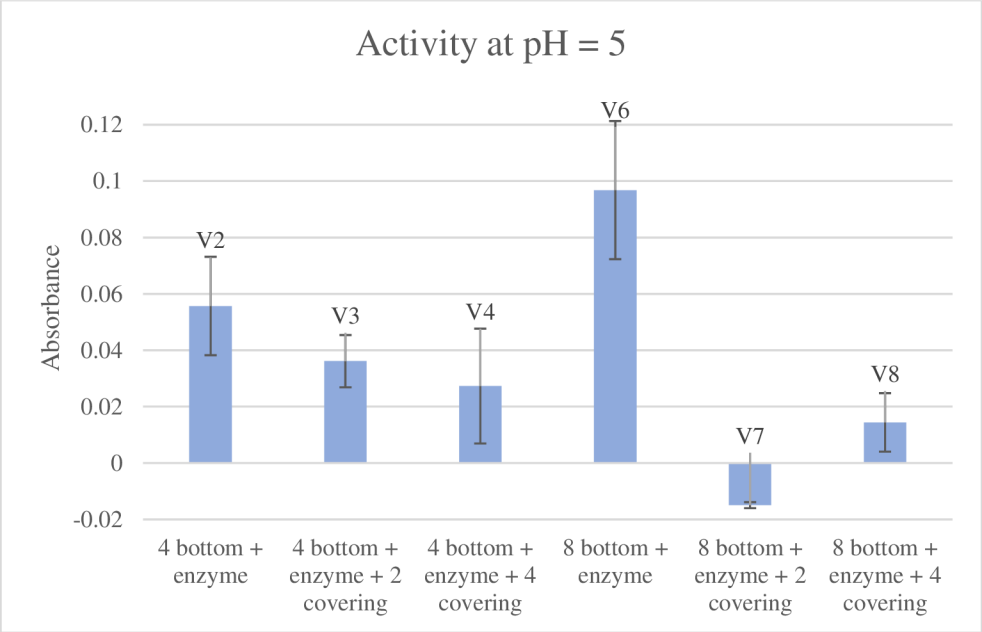

Figure 7: Activity of HRP after addition of TMB; measured using a spectrophotometer at 370 nm; pH = 5 of TBS used in the construction of the layers; All values have been normalized by subtracting the value of the negative controls (V1 and V5).

When lowering the pH of the buffer used to dissolve the polyelectrolytes and the HRP from pH 7.5 to pH 5, the activity of the HRP was lowered. At pH 7.5, the highest measured absorbance after adjustment for the blank was 0.397 in V6. Meanwhile at pH 5, in V6 the absorbance was 0.096. All other samples showed similarly large decreases.

4.2.2 pH = 9

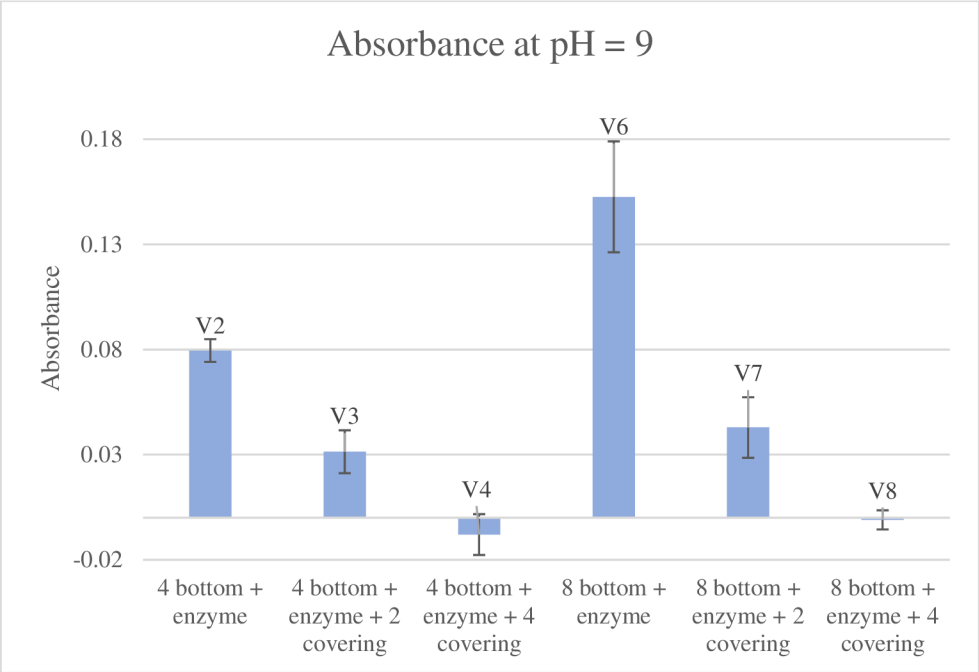

Figure 8: Activity of HRP after addition of TMB; measured using a spectrophotometer at 370 nm; pH = 9 of TBS used in the construction of the layers. All values have been normalized by subtracting the value of the negative controls (V1 and V5).

At pH 9, the activity is lowered as well in comparison to the standard condition of pH 7.5. In V8 the absorbance of -0.001 is barely visible in the graph. This and the negative absorbance in V4 are results due to a contamination of the negative control (V1 & V5) with HRP which has been used to adjust the values. The contamination is also visible in the immunoblotting (Fig. 12), as the layers constructed in this experiment have been used to produce the samples for the SDS-PAGE. Hence an overall higher absorbance than shown in Fig. 8 is likely, which would suggest a higher absorbance than present using the buffer at pH 5.

4.2.3 SDS 1%

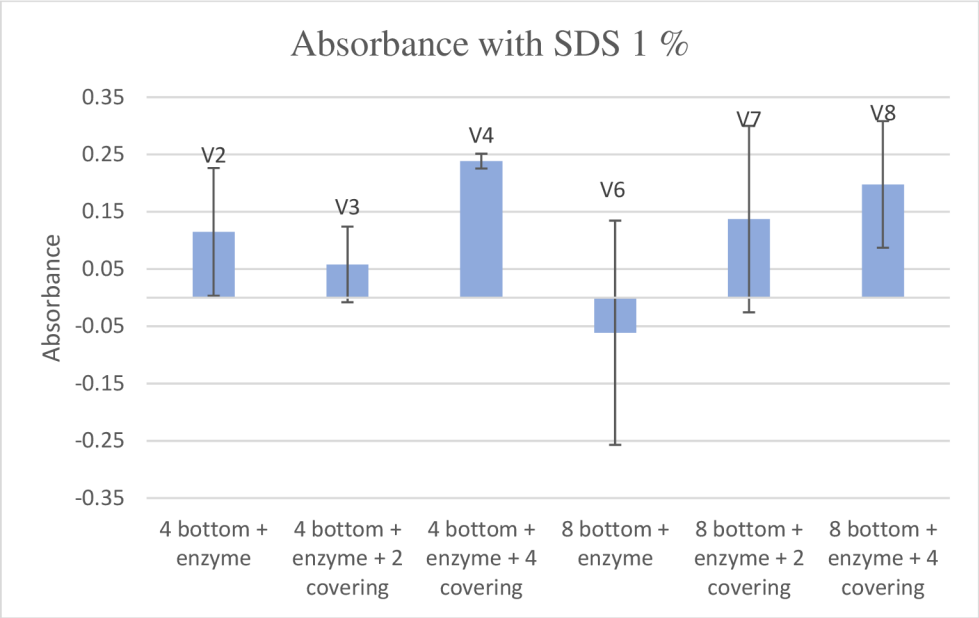

Figure 9: Activity of HRP after addition of TMB; measured using a spectrophotometer at 370 nm; 1 % SDS added to TBS used in the construction of the layers. All values have been normalized by subtracting the value of the negative controls (V1 and V5).

Adding 1 % SDS to the buffer solution caused all polyelectrolyte solutions to be turbid and difficult to filter through the 0.22 µm syringe filters. The turbidity was only partially reduced by filtering the solution and washing of the layers. After adding TMB the resulting blue solution was again turbid which influenced the activity measurement. Nonetheless, the absorbance is lower than in the standard conditions, with the highest absorbance being 0.238 as opposed to 0.397.

4.2.4 SDS 0.1 %

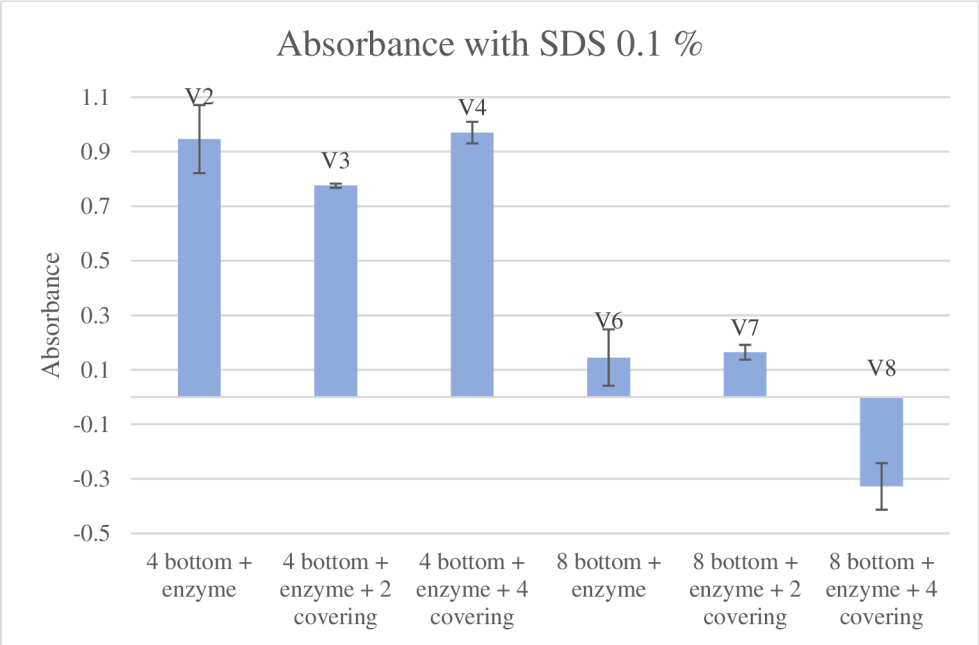

Figure 10: Activity of HRP after addition of TMB; measured using a spectrophotometer at 370 nm; 0.1 % SDS added to TBS used in the construction of the layers. All values have been normalized by subtracting the value of the negative controls (V1 and V5).

The same problems occurred when adding 0.1 % of SDS to the buffer and the high turbidity resulted in only poor measurements. The absorbance seems to be almost doubled in comparison to standard conditions with 0.970 being the highest absorbance vs. 0.397. However, a deactivation of the enzyme and a decrease in absorbance would be expected.

4.3 SDS-PAGE

Figure 11: polyacrylamide gel produced with samples from a layer construction at pH = 9; V1 and V5 are negative controls with contamination. V1 – V4 have 4 base polyelectrolyte layers, V5 – V8 have 8 base polyelectrolyte layers. Each of these sequences has no enzyme and 0, 2, or 4 covering layers above the enzyme respectively. TCE stained and analyzed under UV light.

SDS-PAGE with consequential immunoblotting was performed to confirm the presence and identity of HRP. The gel contained TCE, therefore a simple analysis using UV light was performed (stain-free). No bands are visible by eye, however using this gel for immunoblotting shows very faint signals.

4.4 Immunoblotting

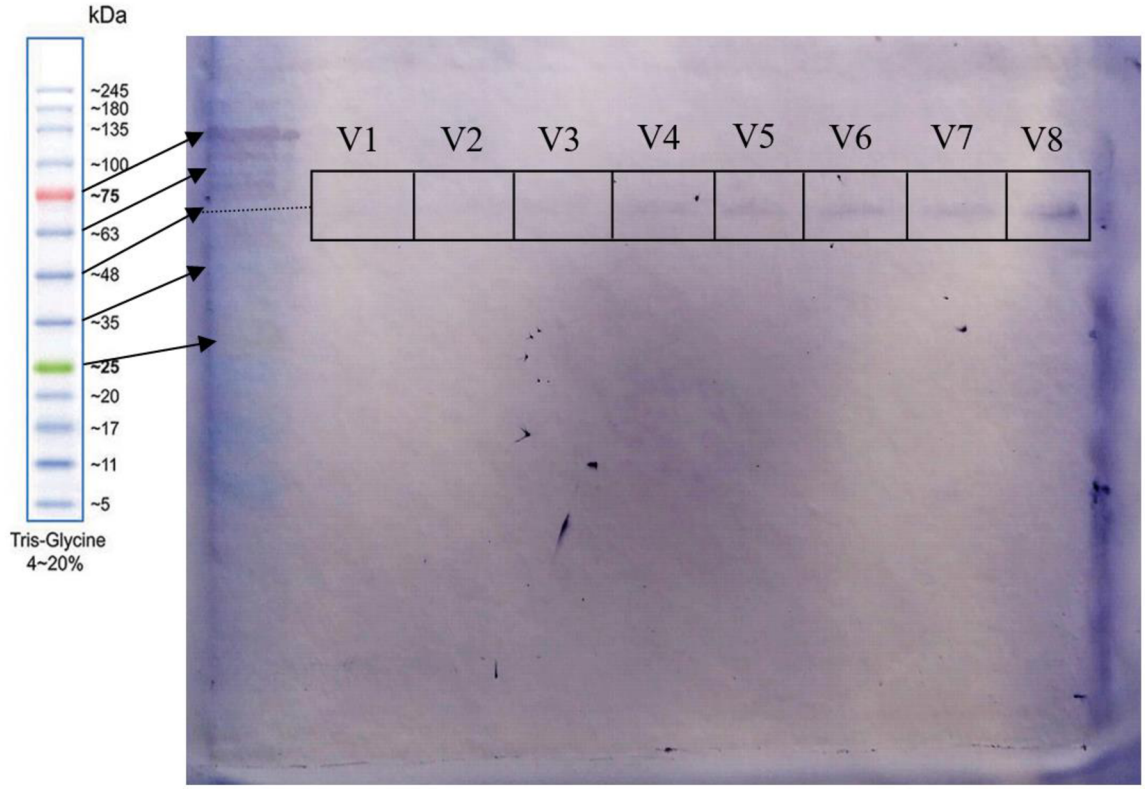

Figure 12: Stained nitrocellulose membrane after immunoblotting. V1 and V5 are negative controls with contamination. V1 – V4 have 4 base polyelectrolyte layers, V5 – V8 have 8 base polyelectrolyte layers. Each of these sequences has no enzyme and 0, 2, or 4 covering layers above the enzyme respectively.

The gel from SDS-PAGE was used to perform immunoblotting. Only weak signals were obtained even after staining overnight. Nevertheless, the stained membrane shows a contamination of wells in V1 and V5 which contained samples without HRP. The gel that was used originated from a layer preparation at pH 9; the corresponding absorbance is shown in Fig. 8 and shows the same problem with the contamination of the negative control. The protein marker in the leftmost well is used to compare the weight of the detected protein. The bands are approximately at a molecular weight of 44 kDa. As the staining was performed using HRP-specific antibodies, this is a confirmation that HRP has been immobilized between the layers.

5. Discussion

An enzyme can be immobilized in numerous ways like adsorption, metal binding, ionic binding, entrapment, and covalent binding [14]. Using polyelectrolytes in the LbL method, multilayers with varying properties can be constructed by changing the conditions. But also the choice of polyelectrolyte can influence the immobilization and the activity expressed by the enzyme.

One of the ways the multilayers are influenced by the properties of the polyelectrolyte is by the molecular weight, which seems to affect the performance and structure of polyelectrolyte multilayers. A low molecular weight (15-20 kDa) polyelectrolyte layer appears to be denser as the short chains have higher mobility, which ensures ideal binding. This mobility also leads to a lower pH stability than in multilayer membranes produced using high molecular weight (150-250 kDa) polyelectrolytes [15]. In this thesis, polyelectrolytes of medium to low molecular weight were used to construct the layers (alternating layers of PSS (Mw = 70 kDa) and PAH (Mw = 17.5 kDa)). Hence, a lower density and sensitivity to pH changes are expected. However, the combination of a strong polyelectrolyte (PSS) and a weak polyelectrolyte (PAH) leads to extremely stable multilayers, withstanding even strongly acidic conditions. The size difference in the polyelectrolyte chains also causes a linear increase of the membrane thickness, as the higher Mw PSS fails to penetrate the small pores of the PAH layers. However, the PSS layers are still compact enough to avoid penetration themselves [15].

Shiratori and Rubner [16] analyzed the average thickness of PAH/poly (acrylic acid) layers in different pH conditions of the solutions. The strongest deposition occurs at pH 5 with a thickness of the PAH layers of ~ 80 Å followed by a sharp decrease. At pH 7.5, the layers were the thinnest at around 4 Å, but at pH 9, the thickness increased again to ~ 50 Å. Increasing the pH above 7 leads to partial deionization of the PAH chain. Naturally, the second used polyelectrolyte contributes to the change in the deposition of PAH as its ionization changes with the pH change and influences the electrostatic interactions [16].

The chosen enzyme for immobilization was Horseradish peroxidase (HRP); a plant enzyme that is stable at pH 5-9, but its optimal pH is slightly below neutral [17]. In experiments with both extracted HRP directly from horseradish roots and purified HRP, the maximum of activity was shown to be from pH 6 to pH 7. Basic environments seem to affect the enzyme more than acidity, the opposite however was observed in the immobilized enzyme in this

thesis. The immobilization of HRP shows a protective effect on the activity compared to free HRP due to the stabilization and protection of the structure [18].

In another study, Anionic glucose oxidase (GOD) was immobilized using PEI and poly (dimethyl diallyl-ammonium chloride). The resulting film was stored in a buffer at 4 °C and maintained the enzymatic activity for 14 weeks. Even raising the temperature to 50 °C showed no significant inactivation of the enzyme, which shows, that a lower susceptibility to temperature changes can be achieved by protection using polyelectrolyte multilayers. The sensitivity to pH changes can also be decreased and a shift of stability towards the basic environment was detected [19].

Releasing the GOD from the multilayers by sonication, and consequent activity measurements showed a decrease of enzymatic activity to 80 % compared to a freshly prepared solution at the same concentration. However, some of this loss can be accounted to the extraction process. Therefore, the rate of denaturation of the immobilized GOD is low [19].

To research the degradation of dyes by immobilized HRP, Saladino et al. [20] produced epoxy-activated acrylic beads conjugated with HRP. They were consequently coated using a total of three layers of PSS and PAH using LbL. The beads were then tested on the effectivity of immobilization and the activity of HRP. Using an incubation time of 24 h of the enzyme, 54 - 59 % activity of the immobilized and covered enzyme compared to the immobilized amount of enzyme was observed. The beads were then used to degrade different dyes and showed on average only about 15 % of the degradation yield after 60 minutes in comparison to free HRP. The activity of the immobilized HRP was observed to reach its peak at around 3 h of incubation and then remained at a constant rate for the 36 h of observed reaction time. After the initial 3 h, the activity of the immobilized and the free HRP are in a similar range. The biggest differences were observed in the first 60 minutes of incubation [20].

Sodium dodecyl sulfate is an anionic surfactant used for cleaning, as a food additive, and in laboratory applications like SDS-PAGE. In this thesis, it was used to analyze the effect of detergents on HRP immobilized by polyelectrolyte multilayers. A too-high concentration of SDS has been used in the experiments by accident. While 1 w% (34.7 mM) and 0.1 w% (3.47 mM) have been produced, 0.01 w% (0.347 mM) and 0.001 w% (0.0347 mM) were desired. It has been shown that a concentration as low as 0.375 mM SDS can decrease the activity of HRP by 62.5 %, the intended concentrations of SDS therefore would have caused different amounts of deactivation. At 0.01 w% SDS, a decrease of ~ 60 % would be expected,

while 0.001 w% SDS should show almost no decrease in activity [21]. In experiments using horse cytochrome c in its reduced state (ferrocyt c), the denaturation of the enzyme starts at ~ 0.1 mM and reaches its maximum at 1 mM [22]. Hence, a denaturation of HRP using the above-mentioned concentrations of SDS is highly likely. The critical micellar concentration of SDS in aqueous environments is ~ 8.5 mM [23], which caused the experienced turbidity during the layer construction and consequent absorbance measurements.

Knowledge of the characteristics of polyelectrolyte multilayers can be used to engineer water and soil treatments for contaminated regions [24] [25], drug-delivery systems in coating for dental implants [26], or tissue engineering [27].

Escherichia coli bacteria can be modified to over-produce atrazine chlorohydrolase. This enzyme degrades atrazine, a commonly used herbicide that has been forbidden in multiple countries but remains in the soil. The cells are modified to be non-viable, as to not disturb ecosystems in the soil, yet they remain active regarding the production of atrazine chlorohydrolase. The cells are then covered using polyelectrolytes, to protect them from the extreme conditions of the soil (pH, temperature, freezing and thawing, predation, etc.) [24].

The bacteria covered with polyelectrolytes showed improved activity compared to untreated cells both in solution and in soil. This is possible, as the surface area is enhanced – allowing for more contact with atrazine – and the outer membrane of the bacteria is disrupted in its configuration by the amine groups of the chosen polyelectrolyte which causes increased permeability [24].

Chloro-organics have been commonly detected in groundwater and soil and have significant impacts on health. To study the degradation of chloro-organics in water, the enzyme laccase was immobilized in pH-responsive polyelectrolyte multilayers. Consequently, the efficiency of dechlorination was tested under a pressure-driven continuous flow mode by allowing the sample to pass through the membrane. In only 36 s of residence time (due to the continuous flow), over 80 % of the initial 2,4,6-trichlorophenol was degraded. Similar results in stagnant water can only be achieved after many hours. The hypothesis was proposed that this is due to the metabolized products quickly leaving the membrane in the flow, avoiding degradation of the enzyme, and fresh reactant can access the active sites. The stability of the immobilized enzyme is also increased, as the free enzyme in solution showed ~ 65 % of activity loss after 22 days, while the immobilized enzyme lost only a miniscule percentage [25].

6. Conclusion

Due to overall time constraints (COVID-19 and consequential halt of all laboratory work for students), many experiments only produced unsatisfactory results, and errors in the procedures could not be found before the data was analyzed. Ideally, most of the experiments would have been performed multiple times with freshly prepared solutions**,** to produce additional data sets and reach a statistical significance of data, while avoiding contaminations introduced in the past.

When adding SDS to the buffer, lower concentrations should have been used and would have most likely produced a solution with less turbidity and consequently more accurate measurements. The presented data is therefore not conclusive on the effect of SDS on the immobilized HRP. This work would have benefited from an additional comparison to the effects of other detergents on the immobilized HRP.

From the produced data, a maximum activity of HRP at neutral pH and with no presence of detergents can be concluded. An immobilization of HRP utilizing multilayers of PSS and PAH produced by the layer-by-layer technique is possible and the activity of the HRP is maintained.

7. Literature references

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