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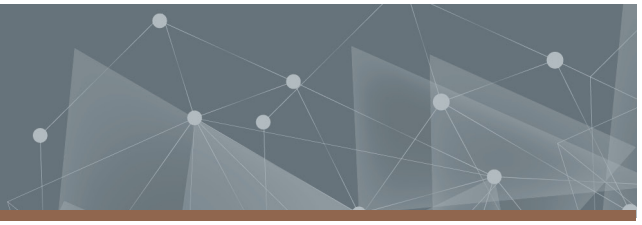

Polyelectrolyte Brushes on Porous Carbon Electrodes for Protein Capture and Electrochemical Release

Master's thesis in Materials Chemistry

ELIN BÅNG

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING

CHALMERS UNIVERSITY OF TECHNOLOGY
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Supervisors: Gustav Ferrand-Drake del Castillo, Oliver Olsson, Nyctea Technologies
Examiner: Andreas Dahlin, Department of Chemistry and Chemical Engineering

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Department of Chemistry and Chemical Engineering
Division of Applied Chemistry
Chalmers University of Technology
SE-412 96 Gothenburg
Telephone +46 31 772 1000

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Abstract

Biopharmaceuticals are medical drugs produced from biological components. Necessary steps in the manufacturing includes isolation and purification of biomolecules, for which chromatography is a common technique. However, it requires harmful elution chemicals, that can affect the desired product. To achieve a more efficient and non-invasive way for biopharmaceutical production, there is an interest of developing new methods for purification of biomolecules.

The electrode surface of Nyctea Technologies utilizes polyelectrolyte brushes responsive to pH-stimuli. By alternating pH, or by electric signals, the brushes can switch between charged and neutral state. This enables interactions that allows the brushes to reversible capture and release biomolecules like proteins. The polymer coating has previous been applied to gold coated steel surfaces. The aim of this project was to investigate alternative substrate materials with the purpose to improve the protein binding capacity of the electrodes. Displaying attractive properties such as high chemical stability and electrical conductivity, having high specific surface area, and high availability at low cost, porous carbon electrode materials was further explored.

The synthesis of poly(methacrylic acid) (PMAA) brushes was performed on reticulated vitreous carbon (RVC) and on carbon felt. By thermogravimetric analysis (TGA) the polymer amount on the coated materials could be quantified. It was confirmed to be PMAA on the felt, while the RVC showed no clear signs of attached polymer. The protein binding capacity of the carbon electrodes was evaluated by pH controlled catch and release of protein, using bovine serum albumin (BSA). The RVC was unsuccessful while the felt preformed better, and was chosen to be further developed as an electrode material. The carbon felt electrodes was assembled in capsules and connected to a chromatography system. Results made clear that protein was captured by the electrodes, and protein release was possible both by pH elution and by electrochemistry, making the carbon felt a promising candidate for future research.

Keywords: Polyelectrolyte brushes, Porous carbon, Protein immobilization, Electrochemistry

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Elin Bång, Gothenburg, June 2024

Contents

1
Introduction

Biopharmaceuticals are medical drugs produced from biological components. They often originate from peptides, proteins, or nucleic acids. Biopharmaceuticals often overcome difficulties seen for conventional pharmaceuticals, like target identification and toxicity from reactive metabolites. Despite attractive features like good biocompatibility, they can be challenging and costly to produce.[1] The manufacture consist of an upstream part, including cell culture and harvest steps. The proceeding part is the downstream process, including purification steps towards the final product. Different chromatography techniques are commonly used for the isolation and purification of biomolecules for the manufacture of biopharmaceuticals.[1] Chromatography separates compounds by their chemical and physical properties. A drawback is the need of large volumes of water and chemicals that risk to create an invasive environment that negatively affects the desired product.[2] The possibility to develop non-invasive and more cost and time efficient methods for biomolecule purification is of interest, and this project has further studied one novel method for this purpose.

The electrode surface of Nyctea Technologies utilizes polyelectrolyte brushes, responsive to pH-stimuli, for purification of biomolecules. By alternating pH, the brushes can switch between charged and neutral state. The switch can also be controlled by electric signals, by applying a potential to the substrate material of the brush electrode. The alternation of the polyelectrolyte charge enables interactions that allows the brushes to reversible capture and release biomolecules like proteins.[3] The polymer coating has previous been applied to gold coated steel surfaces, for successful capture and release of proteins. Gold acts as a great electrode materials, but is expensive and has limited availability. The identification of materials with high electrical conductivity, wide potential window, high surface area and chemical stability is desired. The implementation of an electrode material with these features could potentially lower the cost and increase the protein binding capacity. This project studied porous carbon materials as a substrate material for the polyelectrolyte brush coating of Nyctea Technologies.

1.1 Aim

The aim of this project is to investigate the ability of applying a polyelectrolyte brush coating on porous carbon materials. The purpose is to potentially lower the cost of manufacturing and improve the performance of the electrodes in the product of Nyctea Technologies. One goal is to increase the total binding capacity by working with porous carbon 3D surfaces.

1.2 Outline of Project

The project will be based on laboratory experiments and continuously supported by literature studies. Polymerization on different porous carbon materials will be performed. These will be evaluated by polymer yield and protein binding capacity. The most promising candidate will be further investigated by polymerizing in flow and testing protein binding capacity in chromatography. As a proceeding step, electrochemistry will be implemented for carbon materials in chromatography.

1.3 Limitations

The project will be limited by a few different parameters. The main limiting factor of this project will be the time period, set to 20 weeks. This requires a selection of electrode materials to be investigated, which will be foams (RVC) and carbon felts. These will be used as substrate materials to produce electrodes with polymer on, which will be limited to PMAA polymer brushes. To evaluate the protein binding capacity of the carbon electrodes, both pH and electrochemical release will be performed. BSA (bovine serum albumin) will be used as a model for protein purification.

1.4 Specification of Issue Under Investigation

During the project the following questions will be examined:

- How will the polymerization of polyelectrolyte brushes perform on porous carbon materials? To what extent will the coating cover the surface area throughout the porous material, and how can it be improved?

- How will the carbon electrodes perform in terms of capture and release of protein?

- In what way and how well can the carbon electrodes be applied in a capsule? How can electrochemistry be implemented to the device?

- How can porous carbon materials be used to become a candidate for the product of Nyctea Technologies, considering capacity, scalability, economic and ethical aspects?

2

Theory

2.1 Polyelectrolyte brushes

Polymers are large molecules which are built up by many smaller units, called monomers.[4] They can take many shapes and can have flexible or more rigid structures. They are formed by a process called polymerization. They can be constructed by polymerization on a substrate material, by either grafting from or grafting to process. Polymer brushes are polymer chains densely formed on to a surface. The high surface density will cause the chains to repel each other and making them take a stretched form.[5]

Weak polyelectrolytes are polymers with a functional group that can switch between a neutral and a charged state. If these form a brush like structure on a substrate, the result will be a surface covered by polyelectrolyte brushes. This type of polyelectrolyte brushes can be responsive to environmental stimuli, like the pH in surrounding solution. However, the pH can be locally switched around the brushes by utilizing electrochemistry.[3]

In this project, PMAA polyelectrolyte brushes was used. The brushes are at a neutral state at pH<pKa. For PMAA, a pH 5 is sufficient to protonate the brush almost completely.[6] At this state, the brushes take a collapsed form and can interact with protein by hydrogen bonding. At pKa<pH<pI, the brush begins to take a deprotonated state, making it negatively charged which enables electrostatic interactions with the protein. At further elevated pH, pH>pI and pH>pKa, the brush gets more deprotonated, which will cause a repulsion between it and the protein. This can be controlled by simply increasing the pH of the surrounding solution. However, by a applying a negative potential, electrons will be added to the system and consume protons, causing the brush to be deprotonated. This results in a local pH gradient around the brush. These concepts can be utilized for capture of protein, and release controlled by either pH or electrochemistry.[3]

2.1.1 Synthesis of polyelectrolyte brushes

The synthesis of polyelectrolyte brushes in this project used a grafting-from method, on substrate materials. Diazonium salt is synthesized and covalently attached to a surface by ascorbic acid initiation. This creates a anchor layer on the substrate. The surface is further brominated by bromoisobutyrate together with triethylamine and DCM. From this point, an atom transfer radical polymerization (ATRP) can be initiated by ascorbic acid.[3]

2.2 Carbon electrode materials

Carbon based electrode materials are widely available at low cost. The combination of high electrical conductivity and chemical stability makes it an interesting electrode material for electrochemical purposes. Porous carbon can function as 3-dimensional electrodes, enabling large surface areas for reactions to happen.[7] Carbon as an electrode material also allows for a variety of redox reactions to happen and a wide potential window thanks to the stability of the electrode.[7]

Carbon felt is a soft and compressible porous material, with high electrical conductivity. The surface area is high, but can be difficult to determine considering its fibrous structure.[8] Reticulated vitreous carbon (RVC) is a carbon foam with open pores and high porosity. Despite being fragile, RVC has a rigid structure, has good chemical resistance, high electrical conductivity and high surface area.[9]

2.3 Thermogravimetric analysis

Thermogravimetric analysis is a technique where the material is exposed to gradual heating.[10] The thermal degradation and its resulting mass loss is displayed against the elevated temperature. From these spectra, the weight of different components in a sample can be determined.

2.3.1 PMAA thermal decomposition

The thermal decomposition of pure PMAA was studied by Ho et al.[11] By TGA, the pattern of the weight change of PMAA upon increasing temperature could be identified. It was reported to consist of two regions, illustrated in Figure 2.1. The first region was seen between 140 ^{o}C and 260 ^{o}C, with a maximum decrease rate around 220 ^{o}C. This region corresponds to the formation of poly-methacrylic anhydride (PMAN) from PMAA (see Figure 2.2), which releases water which causes the weight loss. The second region appeared between 300 ^{o}C and 450 ^{o}C, with a much higher decrease rate with a maximum around 410 ^{o}C. This weight decrease was due to loss of carbon dioxide and carbon monoxide at the decomposition reaction of PMAN. After the second region the weight remained constant. The thermal decomposition pattern of PMAA was further confirmed by Andrade-Melecio et al.[12]

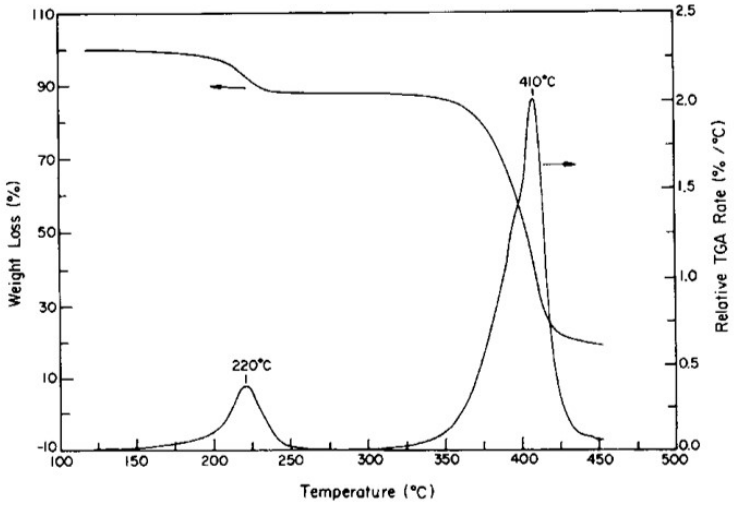
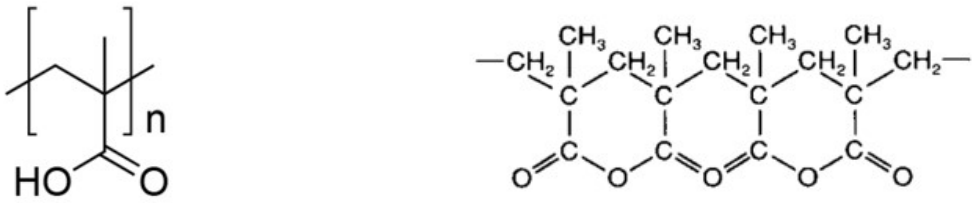


Figure 2.1: Thermal decomposition pattern of PMAA.[11]



(a) **(b)**

Figure 2.2: Molecular structures of (a) PMAA and (b) PMAN.[11]

2.4 Chromatography

Chromatography is a separation method that can separate components by properties such as affinity, size exclusion, ion exchange, and more.[13] Different types of columns can be connected to a chromatography system, and the separation process can be monitored by parameters like UV absorbance, pH, and conductivity. The separation takes place in a column, which holds a stationary phase, where the mobile phase runs through together with the sample to be separated. The components of the sample will interact differently with the stationary and the mobile phase based on their properties and will be separated thereby.[14]

2.5 Electrochemistry

2.5.1 Electrochemical cell

An electrochemical cell consists of electrodes and an electrolyte liquid. A common setup is the three-electrode cell, including a working electrode, a counter electrode,

and a reference electrode. Potential and current can be applied and measured between two electrodes, using an instrument called potentiostat. The working electrode is where the reaction of interest is happening, and should be placed without contact with the counter electrode, which completes the circuit.The reference electrode holds a constant potential to act as a reference to the changes in the system.[15]

2.5.2 Cyclic voltammetry

By cyclic voltammetry (CV) electrochemical properties of a material could be studied. CV is performed by connecting an electrochemical cell to a potentiostat and applying potentials to the system. The change of the current can be analyzed and used to interpret ongoing reactions.[16]

3

Experimental

This chapter provides a description of the experimental procedure of the project. It includes the synthesis method, characterization techniques, and calculations.

3.1 Materials and Chemicals

Of the used chemicals in this project, DCM and DMSO was purchased from Fisher Scientific. All other chemicals were purchased from Sigma-Aldrich. The carbon felt was purchased from FuelCell Store and the RVC from Goodfellow. For the initial syntheses, the electrodes (shown in Figure 3.1) were sized to about 10x10x1 mm. For the continued experiments the carbon felt was cut to cylinders with the diameter 14.5 mm and the height 10 mm.

(a) **(b)**

Figure 3.1: Thin slices of (a) carbon felt and (b) RVC.

3.2 Polyelectrolyte brush synthesis

The synthesis of the PMAA polyelectrolyte brushes on the carbon materials was performed in four main steps; diazonium salt deposition, bromination, polymerization and deprotection. The reaction steps of the synthesis is illustrated in Figure 3.2. The electrodes were prior to the experiment cleaned with SDS (2 %), MQ-water, and isopropanol and dried with nitrogen. After each process step of the synthesis, they were cleaned with ethanol and completely dried with nitrogen gas.

The diazonium salt was synthesized from 0.22 g 4-aminophenethyl alcohol, 1 ml MQ-water, 0.3 ml tetrafluoroboric acid, and put on ice while adding 0.2 ml tert-butyl nitrite, followed by one hour reaction. The adhesion of the diazonium salt

onto the carbon electrodes was done in a 20 min deoxygenated solution of 0.032 g L-ascorbic acid in 45 ml MQ-water, during stirring for one hour. To immerse the electrodes in the ascorbic acid, there was a need to get them wet, remove bubbles, and make them sink below the surface level. This was done by ultrasonication and also by using a syringe for flushing the liquid through the electrodes.

The electrodes were transferred to the bromination step. The reaction was carried out for 15 minutes in a solution of 45 ml DCM, 0.675 ml triethylamine, and 0.5 ml α-bromoisobutyryl.

For the third step, the polymerization, a solution of 20 ml DMSO, 20 ml tert-butyl methacrylate, 0.056 ml N,N,N',N",N"-pentamethyldiethylenetriamine, and 0.006 copper(II)bromide was prepared. With the electrodes inside, the solution was deoxygenated for 30 minutes. The polymerization was initiated by adding 0.5 ml of a solution of 0.06 g L-ascorbic acid in 1 ml DMSO that had been dexoygenated for 10 minutes. The reaction was carried out for one hour.

For the final deprotection step, the electrodes were to react for 15 minutes in 15 ml DCM together with 0.15 ml methane sulfonic acid.

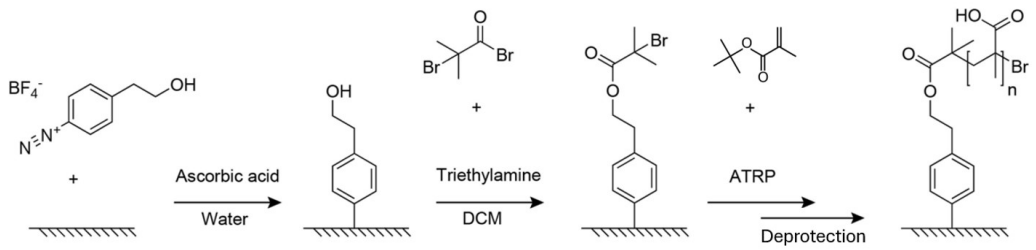
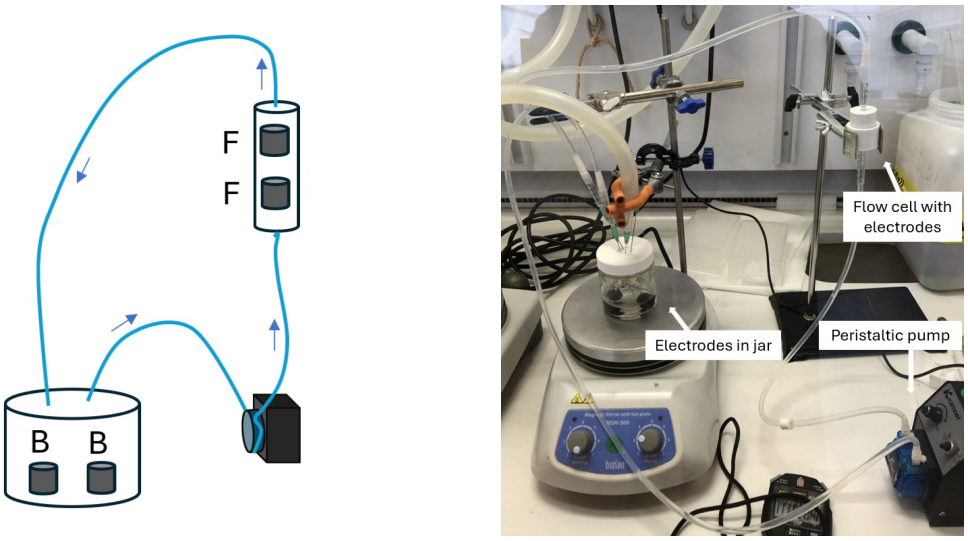


Figure 3.2: Reaction steps of the PMAA brush synthesis.[3]

3.2.1 Synthesis in flow

With the aim to increase the polymer mass surface coverage, the synthesis was developed to have some of the process steps in flow. By pumping the solvents, the purpose was to achieve easier removal of bubbles, improved wetting and reactant exchange inside the porous carbon. A flow cell was designed and 3D-printed (by an Original Prusa SL1S SPEED) to fit two felt electrodes, and two electrodes in a jar were polymerized in parallel as a reference. The flow and the bulk samples were exposed to the same reagents by the setup illustrated in 3.3, to improve the comparability. As shown in Figure 3.4, the bromination and the deprotection steps were not performed in flow, due to avoid pumping the hazardous DCM. Also, the flow cell material would not be DCM resistant.



(a) (b)

Figure 3.3: (a) Simplified schematic of (b) the experimental setup for the synthesis in flow.

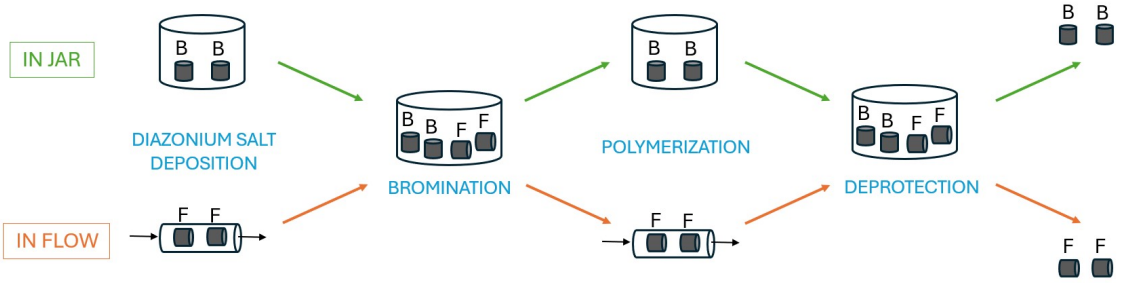


Figure 3.4: Process steps in the flow synthesis of PMAA brushes.

3.2.2 Polymerization during reduced stirring

In contrary to the synthesis in flow, it was of interest to do an experiment at opposite conditions. The synthesis was performed in a jar according to the original method in section 3.2, with the variation of limiting the magnetic stirring during the polymerization step. This was done by two different techniques. The first one was done by stirring only when adding the initiation solution until the color change was visible (maximum 5 seconds), and then leaving it unstirred for the remaining polymerization time. For the second technique, the initiation solution was carefully added by dripping it (for maximum 15 seconds), only relying on diffusion. The two techniques are hereafter referred to as (E) No stirring and (D) Diffusion. To get a good reference sample, electrodes from the same batch was also polymerized during stirring. This is referred to as (C) Stirring, but would practically be the same as the bulk samples.

3.3 Characterization

3.3.1 Thermogravimetric analysis

For this project, TGA measurements was done to quantify the polymer amount on the carbon electrodes. The instrument used was a METTLER TOLEDO TGA/DSC 3+. The measurements was performed in a nitrogen flow of 50 ml/min, with a heating rate of 10 °C/min from 30 to 1000 °C.

The cylinder shaped felt electrodes was prepared for TGA as shown in 3.5. With the purpose to see the polymer coverage throughout the electrode's volume, it was divided in layers from the surface into the center. The RVC samples were smashed into a powder to fill up the measuring crucibles with enough mass for detection.

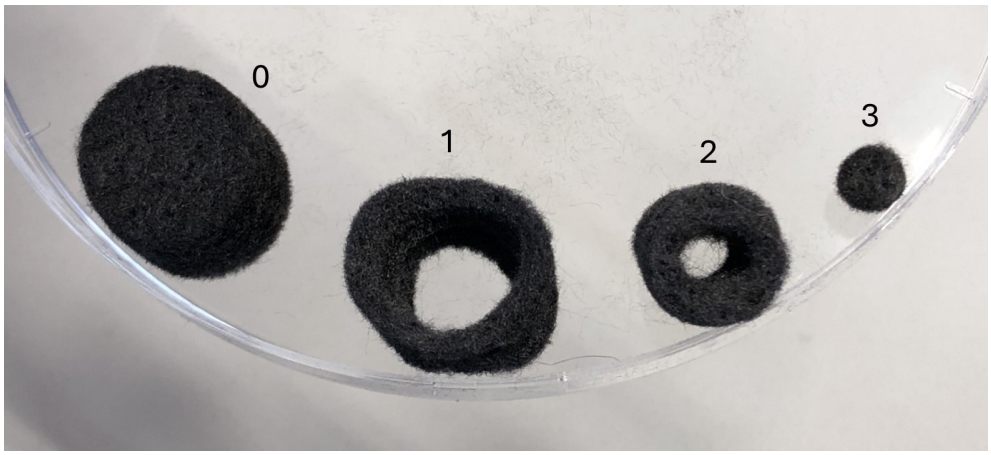


Figure 3.5: The coated carbon felt electrode (0) divided in three layers; (1) surface, (2) second layer and (3) center.

3.3.2 Polymer coverage on carbon electrodes

The polymer amount on the carbon electrodes could easily be quantified as a density; polymer mass per carbon electrode volume. With the carbon felt as example, it can be formulated as

$$Density = \frac{m_{polymer}}{V_{felt}} \tag{3.1}$$

where

$$V_{felt} = \frac{m_{felt}}{\rho_{felt}} \tag{3.2}$$

and ρ_{felt} was determined by weighing a large piece of known volume. However, there is also of interest to find out how well the polyelectrolyte brushes covers the electrode surface area, which was further investigated for the felt. The surface area per volume for the felt was measured with electrochemical impedance spectroscopy (EIS) by Hao Li, resulting in the value A/V = 114.8 cm^2/cm^3. By assuming this is an accurate number, the surface area of the felt was determined by

$$A_{felt} = V_{felt} \times A/V \tag{3.3}$$

and the polymer surface coverage on the felt as

$$Surface\ coverage = \frac{m_{polymer}}{A_{felt}} \tag{3.4}$$

By measuring the volume and mass of a large piece of felt, and using the density of graphite, the felt porosity (Φ) was approximated by

$$m_{felt} = V_{felt}(1 - \Phi)\rho_{graphite} \tag{3.5}$$

and determined to 96.5 %.

3.3.3 Protein catch and release by pH control

The protein binding capacity of the electrodes was examined using bovine albumin serum (BSA). The protein was dissolved in phosphate buffered saline (PBS) of concentrations 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride. The buffer was set to pH 5 for the immobilization step and to pH 7 for the release step. The pH controlled catch and release was performed both in beaker as well as in a chromatography capsule. For the beaker experiments, BSA concentrations was measured by absorbance spectroscopy in a NanoDrop One/One^C instrument.

3.3.3.1 Catch and release in beaker

The electrode was transferred between different beakers with buffer according to Figure 3.6. The electrode was immersed in a pH 5 buffer solution of 2 mg/ml BSA, followed by a rinsing step to remove unbound protein. For the last step, the pH was elevated to 7, with the purpose to release the protein. For each step the BSA concentration was measured. At the immobilization step, the captured protein amount is determined as an indirect value, by calculating the difference between the starting amount and the amount left in the solution after removal of the electrode.

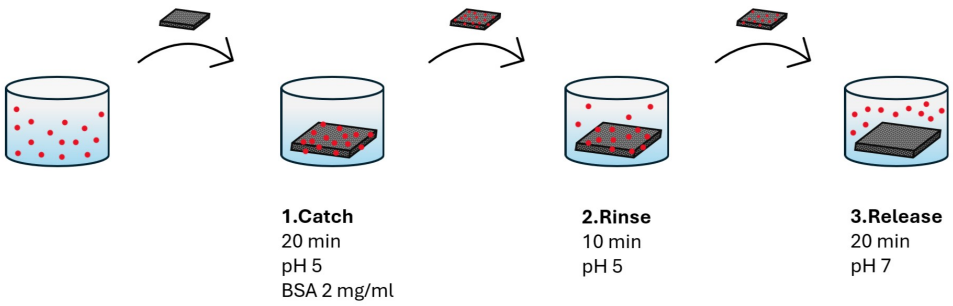
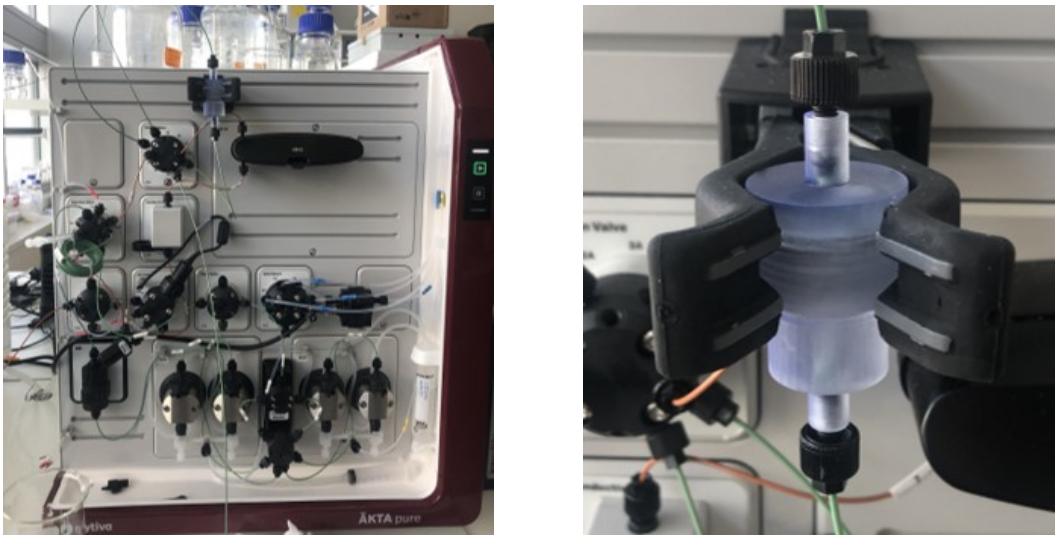


Figure 3.6: The steps of the protein catch and release process, where the carbon electrode is transferred between different beakers after each specified time.

3.3.3.2 Catch and release in chromatography

The protein binding capacity for the carbon felt electrodes was also evaluated in Cytiva's ÄKTA pure™ chromatography system, shown in Figure 3.7a). For this purpose, a capsule (see Figure 3.7b) was designed and 3D-printed in an Original Prusa SL1S SPEED 3D printer. The running buffer was PBS pH 5 followed by the pH 7 elution buffer. A sample of 4 mg/ml BSA was injected and loaded during a 0.1 ml/min system flow. Thereafter the flow rate was increased to 1 ml/min. Three different wavelengths were measured in the experiment, where 280 nm were to be further analyzed.



(a) **(b)**

Figure 3.7: (a) The chromatography system and (b) the connected capsule with carbon felt inside.

3.3.4 Protein catch and release by electrochemistry

The immobilization of BSA was performed by the same method as in section 3.3.3, while the release step was controlled by electrochemistry in either beaker or in capsule.

3.3.4.1 Catch and release in beaker

To prepare the electrochemical experiment the carbon electrode got loaded with BSA according to step 1 and 2 in Figure 3.6. For the release step an electrochemical cell was set up in a beaker, filled with pH 5 buffer. The cell included a working electrode (carbon felt), a counter electrode (stainless steel mesh), and the reference electrode (Ag/AgCl), shown in Figure 3.8. By connecting the electrodes to a potentiostat, a potential could be applied to the system.

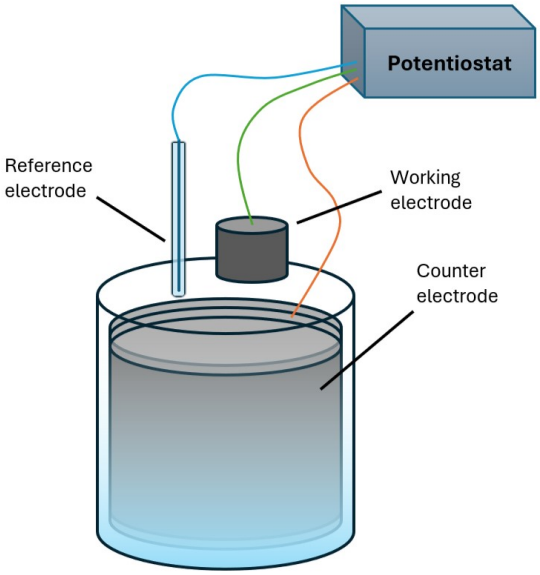


Figure 3.8: Setup for electrochemical experiment in beaker. The working electrode, the counter electrode, and the reference electrode are all connected to a potentiostat.

The procedure was performed in three steps, explained in Figure 3.9. Sampling was done for all steps and the sample liquid was put back to the system after each measurement, to minimize the sample loss. For the electrochemical release, a potential of -1.2 V was applied. After 10 minutes, the working electrode was removed from the electrolyte while the potential was still applied. Hence, the BSA concentration left in the beaker could be quantified, as well as the quantity remaining on the working electrode. The carbon electrode was immersed in pH 5 buffer for a preceding 10 minutes rinsing step, to remove unbound BSA. Thereafter followed a pH controlled release step, leaving the carbon electrode in elution buffer (pH 7) for 20 minutes. All steps described above was performed during stirring by a shaking plate.

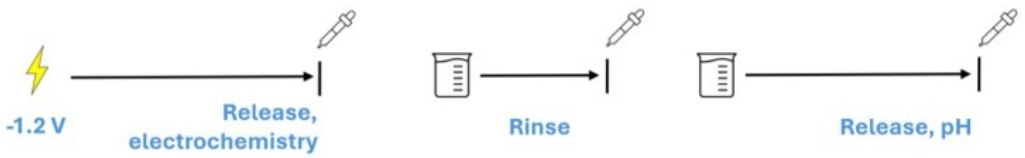
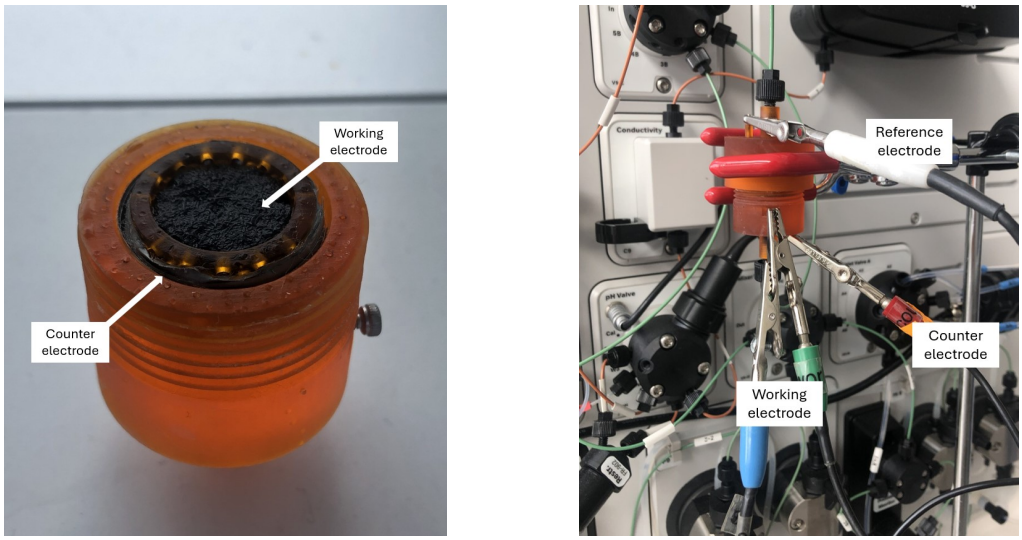


Figure 3.9: Procedure for electrochemical release in beaker. A potential of -1.2 V was applied, followed by a rinsing step before a pH controlled release step. Protein concentrations were measured after each step.

3.3.4.2 Catch and release in chromatography

To transfer from beaker level to chromatography, a new capsule was designed and 3D-printed to fit components needed for electrochemistry. Shown in Figure 3.10a, the working electrode (carbon felt) and the counter electrode (stainless steel mesh) were separated by a wall with holes, to allow electrolyte flow. An Ag/AgCl wire acted as reference electrode, attached in the capsule lid. The capsule was connected to Cytiva's ÄKTA pure™ chromatography system and run by the same settings as in section 3.3.3.2. However, instead of using an elution buffer, the capsule electrodes was connected to the a potentiostat (see Figure 3.10b). By CV-scans of 3 minutes each, potentials were applied as follows; A: -0.7 to -0.8 V, B: -0.9 to -1 V, C: -1.1 to -1.2 V, D: -1.3 to -1.4 V, and E: -1.5 to -1.6 V. The experiment was finished by running the elution buffer (pH 7) to quantify the amount of BSA that didn't got released by electrochemistry.



(a) **(b)**

Figure 3.10: (a) Inside of the capsule bottom. (b) Capsule connected to the chromatography system, and the electrodes connected to a potentiostat.

4

Results and Discussion

This chapter provides a presentation of the results of coating carbon electrode materials with polyelectrolyte brushes. It includes the quantification of the coating and its performance in terms of protein binding capacity controlled by pH shift and electrochemistry respectively, together with an interpretation and discussion of the results.

4.1 Quantification of polyelectrolyte brushes

4.1.1 Comparison of RVC and felt

PMAA brushes were synthesized on 1 mm thick pieces of RVC and carbon felt respectively. Figure 4.1 compares the TGA spectra of RVC and felt. The coated RVC does not show any significant weight change compared to the uncoated reference sample (R). For the coated felt a clear weight drop is visible. The transformation of PMAA to PMAN is noticed around 200 °C by a small weight decrease. Also seen is the large decrease beyond 400 °C, corresponding to the decomposition of PMAN. This confirms there is polymer on the felt, while the RVC doesn't show any typical patterns of polymer decomposition.

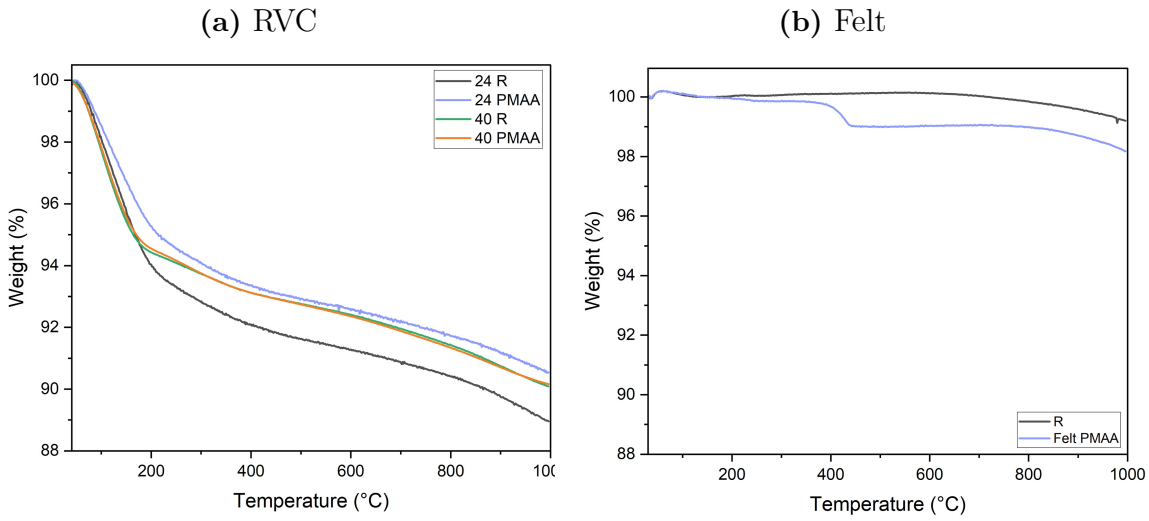


Figure 4.1: (a) TGA spectrum of PMAA coated RVC together with an uncoated reference (R). Two different porosities were tested (24 ppi and 40 ppi). b) PMAA coated felt together with an uncoated reference (R).

4.1.2 Comparison of felt electrodes

Cylindrical felt electrodes prepared in parallel bulk and flow synthesis are compared in Figure 4.2. All samples displays the characteristic decomposition pattern for PMAA. In general, all the bulk electrodes show a larger weight drop than the flow electrodes.

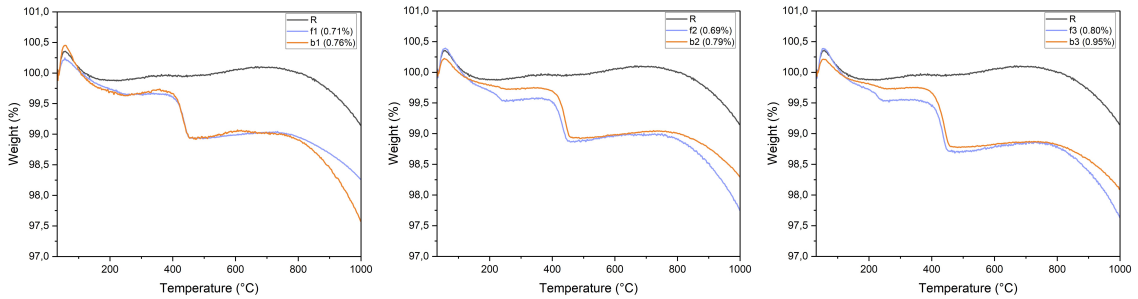


Figure 4.2: TGA spectra comparing (b) bulk and (f) flow synthesized electrodes, as well as an uncoated reference (R). The layers (1) surface, (2) second layer, and (3) center are also compared. The labeled percentage corresponds to the weight drop for each sample.

Felt electrodes polymerized during stirring, no stirring, and diffusion, are presented in Figure 4.3. The stirring and no stirring samples lie within the similar weight decreases, while the diffusion sample appear to have the largest polymer amount. The distribution between the layers will be further presented and discussed in the next section.

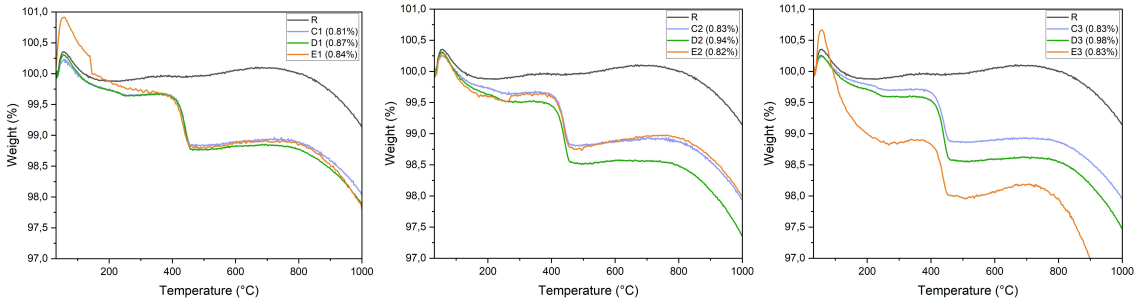


Figure 4.3: TGA spectra of three different electrodes; (C) stirring, (D) diffusion, and (E) no stirring, as well as an uncoated reference (R). The layers (1) surface, (2) second layer, and (3) center are also compared. The labeled percentage corresponds to the weight drop for each sample.

4.1.3 Polymer distribution and coverage on felt electrodes

From the TGA data, the polymer mass for each electrode could be quantified. With the equations in section 3.3.2 the mass per felt volume as well as the mass per felt surface area was determined. The results and the distribution between the layers are presented in Figure 4.4 and Figure 4.5.

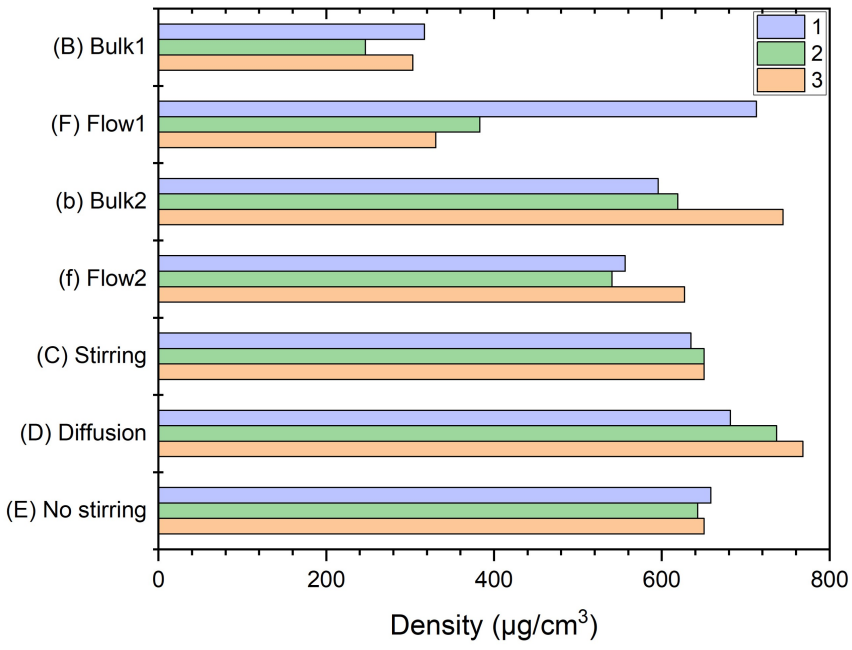


Figure 4.4: The amount of polymer (µg) per carbon felt volume (cm^3). Each sample is divided into three layers, going from the surface (1) to the center (3).

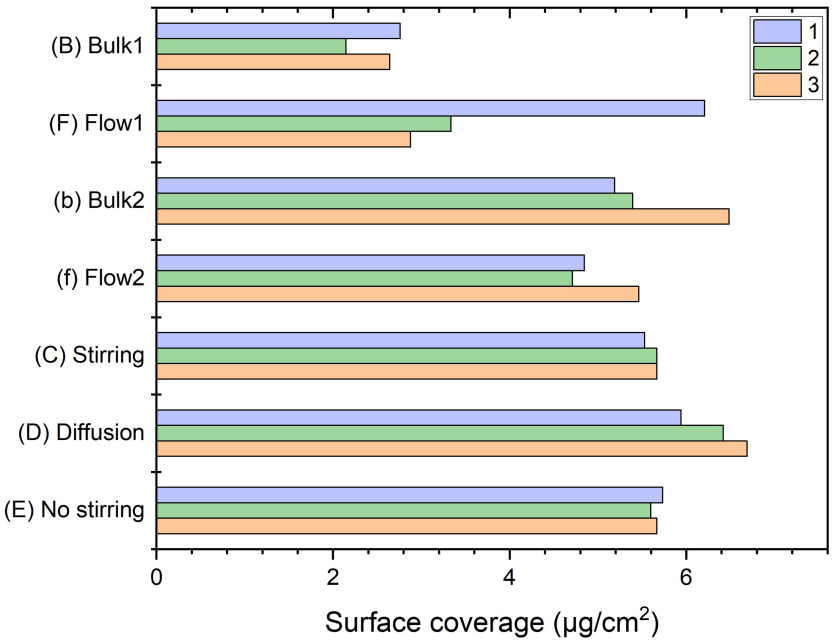


Figure 4.5: The amount of polymer (µg) per carbon felt surface area (cm^2). Each sample is divided into three layers, going from the surface (1) to the center (3).

To start with, the results show that the repeated bulk vs flow experiment (b and f), gained more polymer than the first experiment (B and F). The B and F electrodes had a 1 mm smaller diameter than the rest of the electrodes, leading to larger free volume in the polymerization capsule. This could explain the outlier F1, since the flow distribution probably was higher along the capsule walls, rather than through the felt, giving more polymer on the felt surface. While for f, the electrode was tighter to the capsule walls, forcing the flow path through the felt and giving more polymer on the inside. However, comparing the two methods, flow synthesis does not particularly seem to be beneficial to achieve a larger polymer amount.

Comparing C (stirred) and D (diffusion), synthesised in the same batch, it seems that the method of the polymerization step might played a part. For D, it seems the polymerization in a still surrounding environment favoured the polymer yield on the felt inside. For C, the constant stirring resulted in a very evenly distribution of polymer throughout the felt. For E, stirred for just a few seconds, the distribution is similar to C, which talks for that no stirring is needed for a quite even polymer yield throughout the material.

4.2 Evaluation of protein binding capacity

4.2.1 pH controlled catch and release of proteins

4.2.1.1 Beaker experiments

The protein binding capacity for two kinds of carbon electrodes were tested. Carbon felt and RVC were both utilized for pH controlled catch and release of BSA in beakers. The felt is able to catch and release a larger amount of protein compared to the RVC. This makes sense considering that the felt confirmed to have polymer on it and the RVC did not. Both electrodes let go of large amounts in the rinse step. This indicates that a considerable part of the captured amount is unbound protein, most likely from the liquid that is carried inside the porous structure of the electrodes. Worth commenting is that the RVC in total seem to release and rinse away a larger amount than what is actually captured. Considering that the total amount of rinse and release should add up to the captured amount, it is suspected that some kind of measurement error occurred for the RVC. However, this beaker experiment was mainly an initial test to try the feasibility of protein binding. It was expected to overcome above mentioned problems by getting the electrodes connected to an in flow chromatography system.

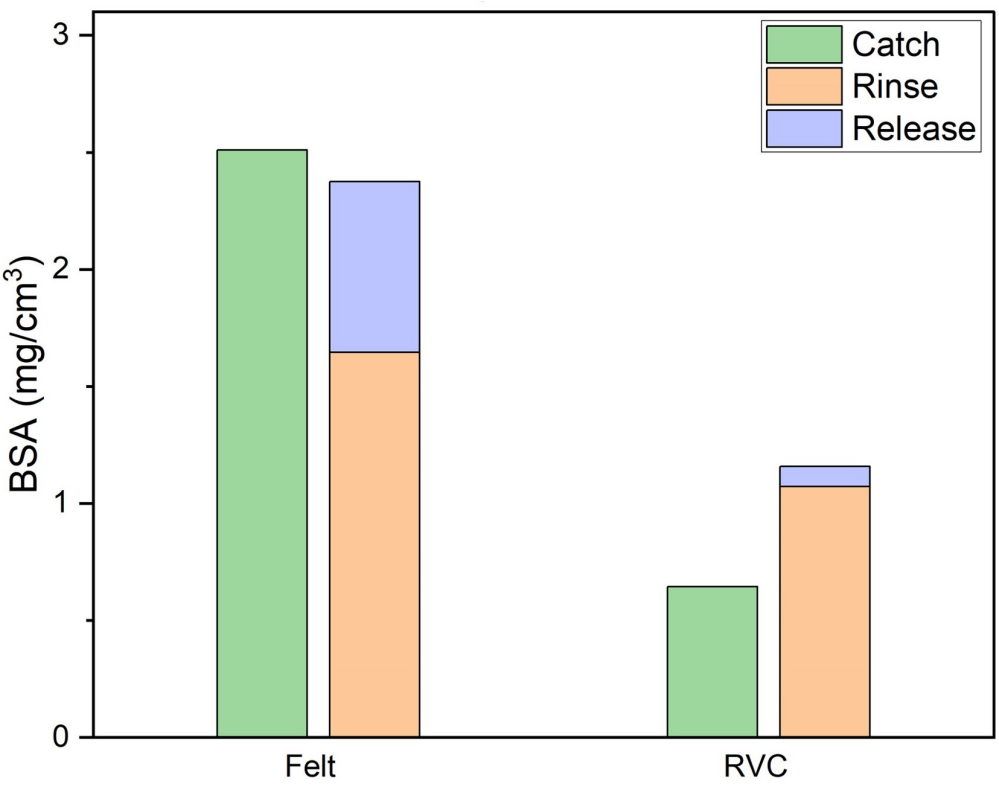


Figure 4.6: Catch, rinse, and released amount BSA per electrode volume for the felt and RVC. The captured protein amount is determined as an indirect value, by calculating the difference between the starting amount and the amount left in the solution after removal of the electrode.

4.2.1.2 Chromatography experiments

The cylindrical felt electrodes were assembled in a capsule and connected to the chromatography system. Figure 4.7 demonstrates the chromatogram of three electrodes; bulk (b), flow (f), and no stirring (E). The small peaks indicates that b and f releases similar amounts, while E is able to release almost the double amount.

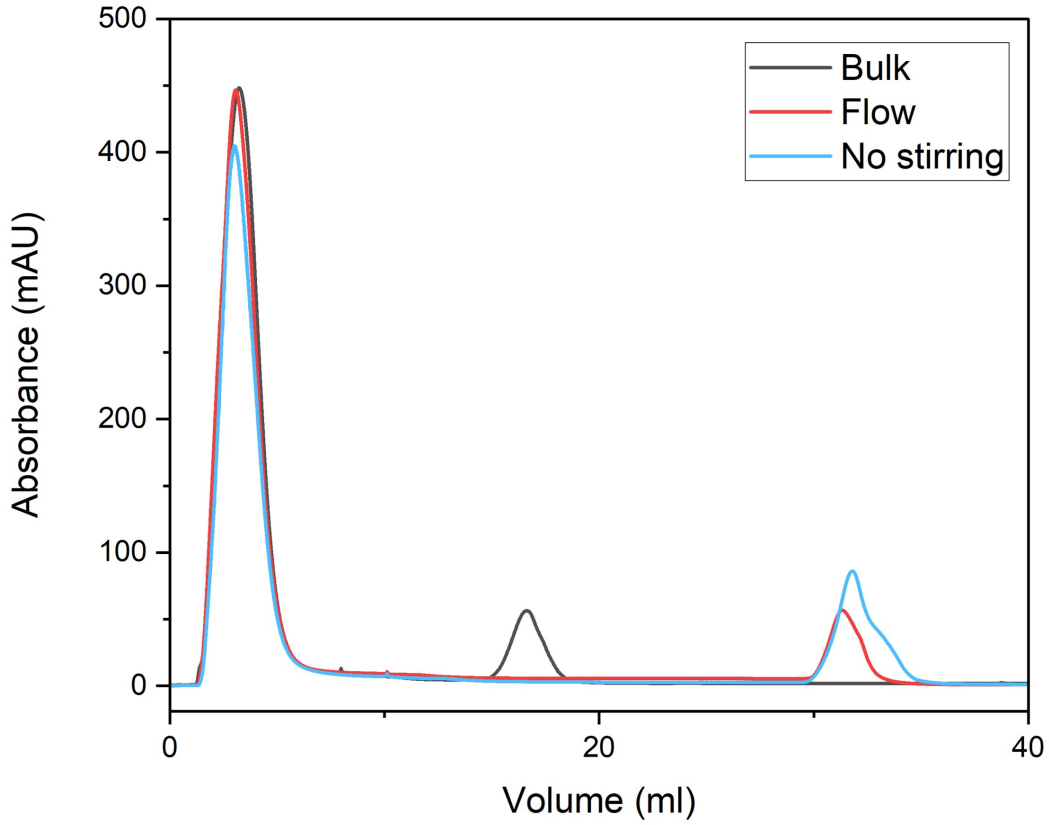


Figure 4.7: Chromatogram (280 nm) of pH controlled immobilization and release of BSA on three different carbon felt electrodes.

Table 4.1 presents the catch and release, received by using the extinction coefficient of BSA (0.67) [17] and the integrated peak area. The protein binding is also evaluated per felt volume and felt surface area. The higher protein binding of E compared to b and f aligns with the results of E having a larger amount of polymer.

Table 4.1: Comparison of three different felt electrodes. Catch and release are presented by amount, per volume, and per surface area. The catch is what was stuck to the electrode (indirect value) and the release is what came out to the solution.

	BSA (mg)		**BSA/volume (mg/cm^3)**		**BSA/area ($\mu g/cm^2$)**	
	Catch	Release	Catch	Release	Catch	Release
Bulk	1.026	0.773	0.667	0.502	5.804	4.373
Flow	1.203	0.767	0.781	0.498	6.806	4.339
No stirring	1.998	1.405	1.193	0.839	10.39	7.308

4.2.2 Catch and electrochemical release of proteins

4.2.2.1 Beaker experiments

The possibility to get electrochemical release of BSA was tested according to the schedule in Figure 3.9. The felt electrode polymerized during stirring (C) was the one used for this purpose. The results in Figure 4.8 confirms that it is feasible, and in addition succeeds to get the most amount of protein released by electrochemistry. After the electrochemical release, and the rinsing step, there is not much protein left that can be released by elevating the pH.

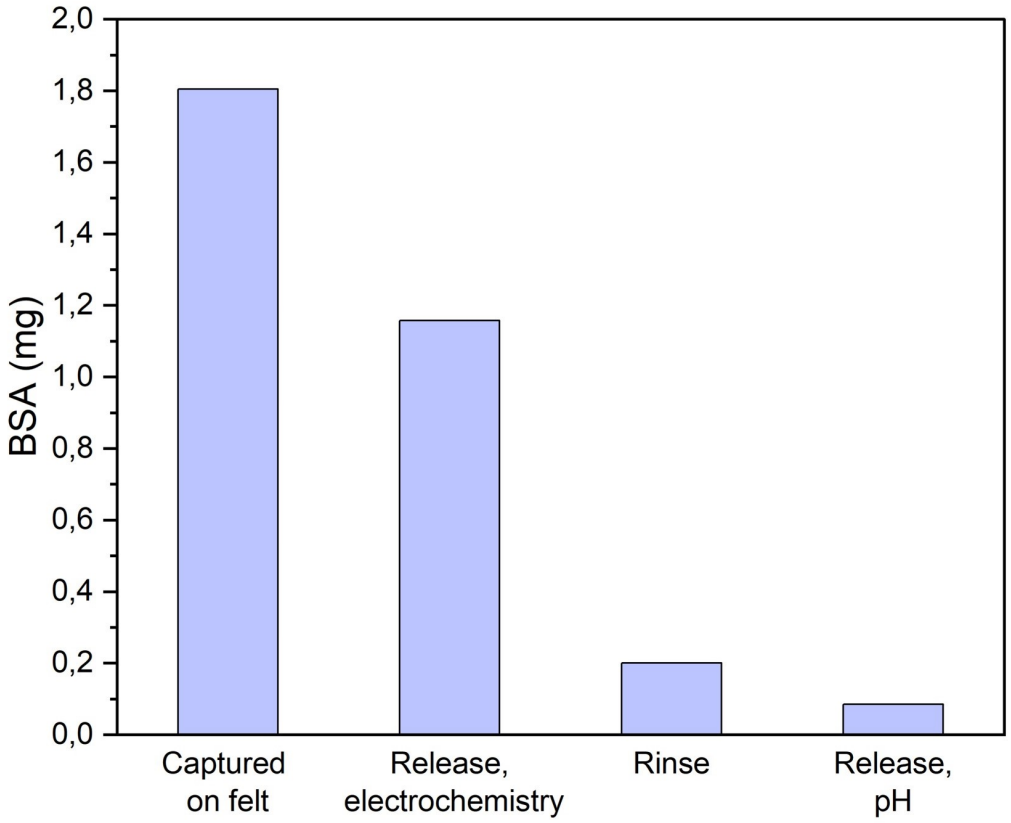


Figure 4.8: Catch and release by electrochemistry in beaker. The captured amount is an indirect value and corresponds to the amount of protein on the electrode. For the release by electrochemistry, the rinse, and the release by pH, the values corresponds to the amount of BSA that is released into the solution.

4.2.2.2 Chromatography experiments

The felt was assembled in a capsule designed for electrochemical components, and connected to the chromatography system. For this experiment the electrode polymerized by diffusion (D) was used. The chromatogram in Figure 4.9 confirms that the pH is elevated while applying a potential, and reaches higher values with increased (negative) potentials. The absorbance curve is drifting upwards, which for the future could be avoided by increasing the time between the peaks, letting the curve settle back to baseline. Higher flow rates (than 1 ml/min) could be another possible improvement, as the protein detection may benefit of higher flow through the felt's porous structure. Despite this, clear increases of the absorbance are seen while the pH is elevated, indicating release of protein. Further, it seems to be almost fully released by electrochemistry, since minimal amounts are released by the pH elution. These results are strengthened by the measured concentrations of the fractionated samples from the experiment. Figure 4.9 (bottom) illustrates how the concentration changes for each of the fraction. Aligned with the chromatogram, it show a concentration pattern with increase at every peak. However, the absolute values showed to be inaccurate, and were instead measured by collecting multiple

fractions over larger spans together. The results for catch and release can be read in Table 4.2.

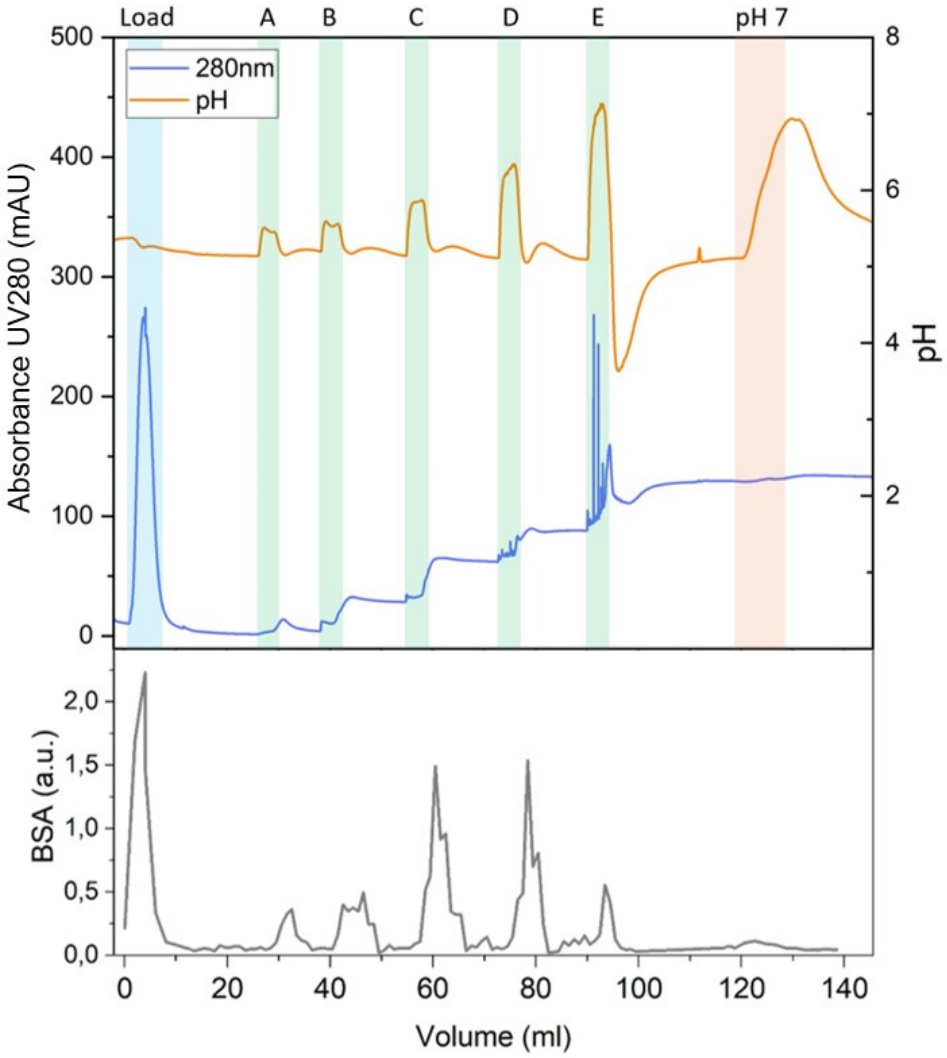


Figure 4.9: Chromatogram of immobilization and electrochemical release of BSA. The sections A-E corresponds to different potentials applied (A: -0.7 to -0.8 V, B: -0.9 to -1 V, C: -1.1 to -1.2 V, D: -1.3 to -1.4 V, and E: -1.5 to -1.6 V).

Table 4.2: Catch, pH release, and electrochemical release of BSA. The catch is the amount that stuck to the electrode (indirect value), and the release is the amount that came out to the solution after applying a potential or increasing the pH. Results are also compared per felt volume and felt surface area.

	BSA (mg)	**BSA/volume (mg/cm^3)**	**BSA/area ($\mu g/cm^2$)**
Catch	3.86	2.31	21,5
Release, electrochemical	3,43	2,05	17,8
Release, pH	0,358	0,214	1,86

5

Conclusion

This project had the aim to create a new type of electrode with reversible protein capturing and releasing capability. The polymerization of PMAA polyelectrolyte brushes was performed on porous carbon materials; felts and foams. By TGA the coated electrodes were analyzed to quantify the gained polymer amount. The RVC didn't display any clear weight changes according to the decomposition pattern. An explanation could be that the surface area of RVC is to small to offer enough space for polymer to grow on.

For the felts however, the characteristic thermal decomposition pattern was visible. Starting with a small weight decrease around 200 ^{o}C, followed by a clear drop around 400 ^{o}C. These results confirmed there was polymer on the felts, and the amount could also be quantified and the distribution throughout the material estimated. The felt electrodes were synthesized by different methods; in bulk, flow, stirring, no stirring, and diffusion. With the purpose to reach larger polymer yields, no obvious benefits could be identified by having the synthesis in flow. Same goes with the methods of having reduced stirring. No significant differences has been proven, and the gained polymer amount seem to be limited by the reaction itself rather than the mass transport.

The protein binding capacity was examined by catch and release controlled by pH. Initial tests in beaker were done to compare felt and RVC. The felt was the best performing for both catch and release. The felt was hereafter decided to further be tested in chromatography controlled by pH. The protein capture and release increased with the electrodes with larger polymer amount on them. At most, one electrode captured 2 mg and released 1.4 mg.

To implement electrochemistry, an initial beaker experiment was performed. The results showed it was possible to get a majority of the captured protein to be released by electrochemistry. A proceeding step was to assemble a capsule and control elution in chromatography by electrochemistry. It was concluded that an applied potential could elevate the pH, and as a result achieve protein release. Also here, the majority of the captured protein could be released by electrochemistry.

The future of the porous carbon materials still face some challenges. It is desired to scale up, establish the most favorable synthesis path, and reach the ability to preform multiple catch and release cycles by the felt electrodes. However it has already shown interesting results at this early stage. By further research and continued development, the carbon felts could potentially be a promising electrode material in the product of Nyctea Technologies.

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