

Fabrication and Modification of Functional and
Enzymatically Active Hollow Fiber Membranes
Herstellung und Modifizierung von funktionellen und enzymatisch
aktiven Hohlfasermembranen

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I still remember a cold afternoon in March 2013, when I arrived in Germany to pursue my university studies. As I looked out of the train window, my thoughts were with my family and the warm weather back home in Colombia. Back then I was convinced that coming to Germany had been a terrible mistake. The person I was then would never, in a million years, have believed that thirteen years later I would be holding a completed PhD thesis in my hands. This achievement, however, would never have been possible without the support and guidance of the many people I met along my academic journey.

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*Two roads diverged in a wood and I - I took the one less traveled by, and
that has made all the difference.*

Robert Frost "The Road Not Taken" (1916)

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Abstract

Enzymatic membrane reactors have emerged as powerful tools for process optimization, integrating biocatalysis with membrane separation. The capacity of enzymes to operate under mild conditions (physiological pH and temperature) can significantly lower energy costs while maintaining product quality. However, enzymes degrade under challenging conditions (e.g., high temperatures and organic solvents), limiting their use in industry-relevant processes.

To enhance enzyme stability in membrane processes, enzymes are typically immobilized on either the membrane surface or within its matrix. Unfortunately, conventional methods rely on harsh solvents and high temperatures, which degrade enzymes, thus limiting immobilization only after membrane formation. Additionally, most commercial membrane materials lack functional groups for effective enzyme attachment, requiring time-consuming post-modification procedures.

This thesis explores innovative techniques for fabricating biocatalytic hollow fiber membranes by directly integrating enzymes and functional materials into the structure, enhancing enzyme stability and introducing new functionalities beneficial to separation processes. Each method improves upon the current state-of-the-art by reducing steps required for enzyme immobilization or by simplifying functionalization. Three methods are discussed:

The first method is the fabrication of free-standing, mechanically stable biocatalytic polyelectrolyte complex (PEC) hollow fiber membranes using salt-dilution induced phase separation. Since this method does not involve organic solvents, enzymes can be directly added to the polymer solution, simplifying immobilization. The PEC membranes are characterized by their separation performance in nanofiltration and enzymatic activity. The second method is modifying hollow fiber membranes via single-step PEC coating—a viable alternative to the time-consuming layer-by-layer process—producing membranes with biocatalytic and nanofiltration properties. The third method is fabricating low-fouling and self-cleaning hollow fiber membranes achieved by immobilizing microgels and enzymes on the surface.

These methods produced biocatalytic hollow fiber membranes with enhanced enzyme stability and activity and additional beneficial properties such as ion selectivity, reduced fouling propensity, and nanofiltration performance. This work demonstrates that integrating enzymes and functional materials into hollow fiber membranes enhances enzyme stability and introduces new functionalities, advancing the development of biocatalytic membrane reactors for industrial and biomedical applications.

Zusammenfassung

Enzymatische Membranreaktoren haben sich als leistungsfähige Mittel zur Prozessoptimierung etabliert und verbinden dabei die Biokatalyse und die Membrantrennung. Die Fähigkeit von Enzymen, unter milden Bedingungen (physiologischer pH und Temperatur) zu arbeiten, senkt die Energiekosten bei gleichbleibender Produktqualität erheblich. Allerdings degradieren Enzyme unter schwierigen Bedingungen (z. B. hohe Temperaturen und organische Lösungsmittel), was ihre Verwendung in industriellen Prozessen einschränkt.

Um die Enzymstabilität in Membranprozessen zu erhöhen, werden Enzyme entweder auf der Membranoberfläche oder in der Matrix immobilisiert. Leider sind herkömmliche Methoden auf aggressive Lösungsmittel und hohe Temperaturen angewiesen, wodurch die Enzyme degradiert werden, sodass die Immobilisierung erst nach der Membranbildung möglich ist. Außerdem fehlt es den meisten kommerziellen Membranmaterialien an funktionellen Gruppen für eine wirksame Enzymanlagerung, was zeitaufwändige Nachmodifizierungsverfahren erforderlich macht.

In dieser Arbeit werden innovative Techniken zur Herstellung biokatalytischer Hohlfasermembranen erforscht, bei denen Enzyme und funktionelle Materialien direkt integriert werden, um die Stabilität der Enzyme zu erhöhen und neue Funktionalitäten einzuführen. Jede nachfolgend untersuchte Methode verbessert den aktuellen Stand der Technik, indem sie die Enzymimmobilisierung beziehungsweise die Funktionalisierung vereinfacht. Es werden drei Methoden diskutiert:

Die erste Methode ist die Herstellung freistehender, biokatalytischer Polyelektrolytkomplex (PEC) Hohlfasermembranen durch salzverdünnungsinduzierte Phasentrennung. Da diese Methode ohne organische Lösungsmittel auskommt, können Enzyme direkt in die Polymerlösung eingebracht werden, was die Immobilisierung vereinfacht. Die zweite Methode besteht darin, Hohlfasermembranen durch eine einstufige PEC-Beschichtung zu modifizieren. Diese Vorgehensweise stellt eine praktikable Alternative zum zeitaufwändigen Layer-by-Layer-Verfahren dar und ermöglicht die Herstellung von Membranen mit biokatalytischen Eigenschaften. Die dritte Methode produziert selbstreinigende Hohlfasermembranen mit geringem Fouling durch Immobilisierung von Mikrogelen und Enzymen auf der Oberfläche.

Diese Methoden ermöglichen die Herstellung biokatalytischer Hohlfasermembranen mit erhöhter Enzymstabilität und -aktivität sowie vorteilhaften Eigenschaften wie Ionenselektivität und Nanofiltrationsleistung. Diese Arbeit zeigt, dass die Integration von Enzymen und funktionellen Materialien in Hohlfasermembranen die Stabilität der Enzyme erhöht und neue Funktionalitäten einführt, was die Entwicklung biokatalytischer Membranreaktoren für industrielle Anwendungen vorantreibt.

Introduction

Synthetic membranes have now become essential contributors to the development of sustainable industrial processes. Membrane processes enable unique separations that other methods cannot achieve as efficiently or selectively, most notably desalination and gas separation [Bake2023]. However, with increasing water scarcity and shifting demands in the energy sector, there is an increased need for even more efficient and creative membrane processes.

Enzymatic membrane reactors have emerged as a powerful tool for process optimization. Enzymes are biocatalysts that can accelerate a wide range of reactions while operating under mild conditions (physiological pH and temperature). However, enzymes are expensive, and their lack of operational stability and difficult recovery make processes involving soluble enzymes costly. Immobilizing enzymes directly on membranes offers a viable alternative, enabling simultaneous reaction and product separation while ensuring enzyme reuse. However, since common membrane materials lack the necessary properties for enzyme immobilization, novel fabrication, and modification techniques are required to facilitate enzyme attachment and enhance membrane properties by imparting additional functionalities [Joch2011].

However, most membranes are made from a limited selection of commercially available polymers, such as cellulose acetate (CA), polyimide (PI), polysulfone (PS), and polyvinylidene fluoride (PVDF) [Bake2023]. However, membrane fabrication with these materials has been extensively researched

and optimized over the past decades, with further improvement mostly stagnated [Gin2011; Nune2020]. New and creative membrane fabrication methods are needed. By employing different fabrication and modification techniques, it is possible to exploit synergies that enhance membrane performance beyond what traditional materials allow. For instance, novel materials, additives, and innovative fabrication methods can enable the integration of sensitive enzymes while improving membrane selectivity and introducing new functionalities that benefit the separation process [Rest2024].

This work explores different fabrication strategies, enzyme immobilization techniques, and the resulting membrane performance in relevant applications. The focus is on developing hollow fiber membranes with biocatalytic properties by integrating functional enzymes into the membrane structure. The thesis is structured as followed:

Chapter 2 provides an overview of various membrane types and structures, along with a classification of the membrane processes relevant to this thesis. It then explores phase separation, covering both non-solvent induced phase separation and aqueous phase separation of polyelectrolyte complexes, followed by a brief introduction to hollow fiber membrane fabrication via dry-wet jet spinning. Additionally, membrane functionalization is addressed through post-modification and additive blending. Lastly, the concept of enzymatically active membranes and an overview of different enzyme immobilization techniques are presented. The subsequent chapters focus on different types biocatalytic functional membranes achieved through different methods. A graphical representation of the chapters is given in Figure 1.1.

Chapter 3 details the successful fabrication of free-standing, mechanically stable biocatalytic polyelectrolyte complex membranes. Since membrane fabrication is conducted via aqueous phase separation, the enzyme alkaline phosphatase (ALP) is easily incorporated by adding it directly to the polymer solution, allowing for in situ entrapment during membrane formation. This approach ensures that ALP remains stable even after multiple filtration cycles while also enhancing the enzyme's thermal and storage stability. In addition to their catalytic activity, the membranes exhibit nanofiltration

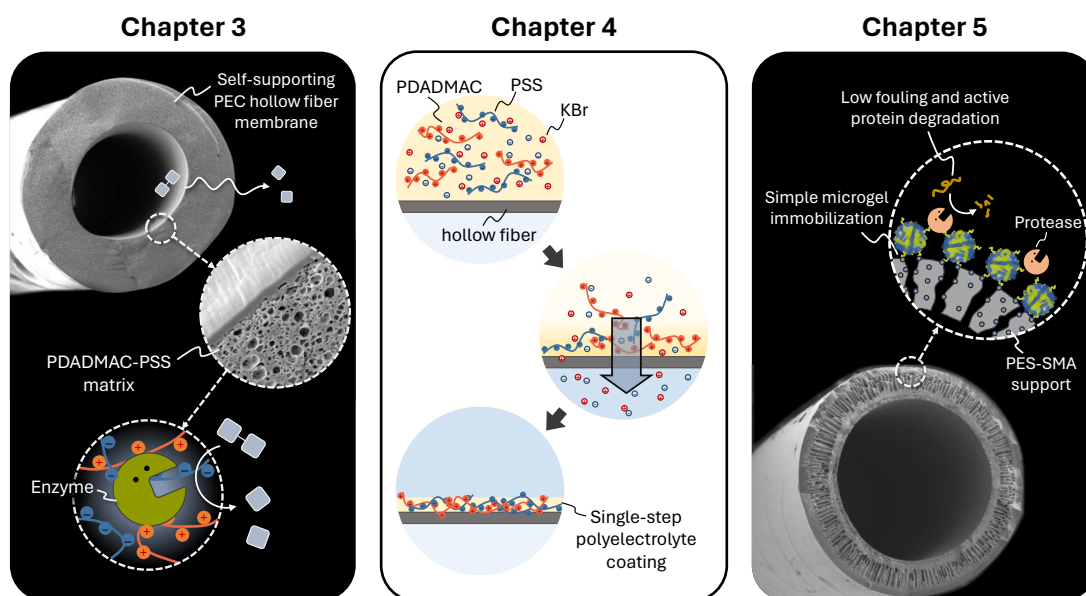


Figure 1.1: Visualization of the types of biocatalytic and functional membranes fabricated and investigated in this thesis.

properties. This work highlights the versatility of the fabrication of polyelectrolyte complex hollow fiber membranes via the salt-dilution induced phase separation, serving as a platform technology for the fabrication of porous, selective and bioactive membranes.

Chapter 4 presents three novel methods for the single-step polyelectrolyte complex coating of hollow fiber membranes. With these techniques, oppositely charged polyelectrolytes can be effectively applied to the hollow fiber surface, eliminating the need for the traditional, time-consuming layer-by-layer (LBL) deposition process. Three distinct coating approaches are presented: Interfacial Complexation (IC), Diffusive Desalination (DDS), and Polyelectrolyte Concentration (PC). While membranes with nanofiltration properties can be fabricated following IC and PC methods, DDS allows for the straightforward immobilization of enzymes. In this case, the model enzyme ALP was also used.

Chapter 5 details the fabrication of low-fouling hollow fiber membranes by immobilizing amine-functionalized microgels onto a blended polyether-sulfone (PES) and styrene maleic anhydride (SMA) support. Hollow fibers are fabricated via conventional non-solvent induced phase separation. Sub-

sequently, membranes are coated with poly(N-isopropylacrylamide)-based microgels enriched with 2-aminoethyl methacrylate hydrochloride (AEMA). The amine groups in AEMA react with SMA under alkaline conditions, ensuring the stable immobilization of the microgels via imide bond formation, thereby enhancing the membrane's hydrophilicity and antifouling properties. In addition, the proteolytic enzyme trypsin is also immobilized on the membrane surface, enabling the active degradation of the foulants. This work highlights the versatility of SMA as a functional blending additive in membrane fabrication and demonstrates the efficacy of combined microgel and enzyme coatings in establishing robust, hydrophilic, and self-cleaning barriers.

Chapter 6 summarizes and reflects the three membrane fabrication methods explored in the thesis. Moreover, the future perspectives of the work are discussed, emphasizing that while challenges like high production costs and limited enzyme stability remain, focusing on processes that can significantly benefit from biocatalytic membranes without relying on long-term enzyme stability—such is beneficial.

2

Fundamentals

Parts of this chapter have been published as:

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2.1 Membrane Processes

A membrane is defined as a semi-permeable structure that allows the selective separation of components dissolved in a fluid (liquid or gas). These components can be dissolved solutes such as ions, proteins, microorganisms, suspended solids, or a mixture of fluids. Thus, selectively retaining a target component can enrich a feed solution with the component while it is depleted from a permeate. However, a driving force must be applied to overcome the membrane barrier. This force can be in the form of a difference in pressure, concentration, temperature, or electrical potential [Bake2023].

Membranes can be classified into three categories, as observed in Figure 2.1: Isotropic, anisotropic, and membranes with special features. Isotropic or symmetric membranes have a uniform chemical composition and structure (porous or dense). On the other hand, anisotropic or asymmetric membranes may have pores of varying dimensions across their structure or consist of layers of different materials (e.g., thin-film composite membranes). The third category encompasses membranes with unique features that enable or facilitate the transport of desired substances. For instance, electrically charged membranes possess fixed charges in their polymeric backbone that attract or repel ions based on their charge. These types of membranes are used in electrically driven membrane processes such as electrodialysis. A second example are mixed matrix membranes. These composite membranes comprise a porous matrix combined with highly selective and permeable filler materials, such as nanoparticles, zeolites, or metal-organic frameworks. These particles can also catalyze reactions occurring during the separation process. The last type of membranes with unique features are liquid membranes. These consist of a porous matrix in which the pores are filled with a liquid that can reversibly bind one of the components in the feed solution, thus enabling its separation [Bake2023].

The type of Membrane structure closely dictates the type of transport mechanism occurring within the membrane. Two basic models are commonly used to describe this transport: the pore-flow model and the solution-diffusion model. In the pore-flow model, convective transport through the

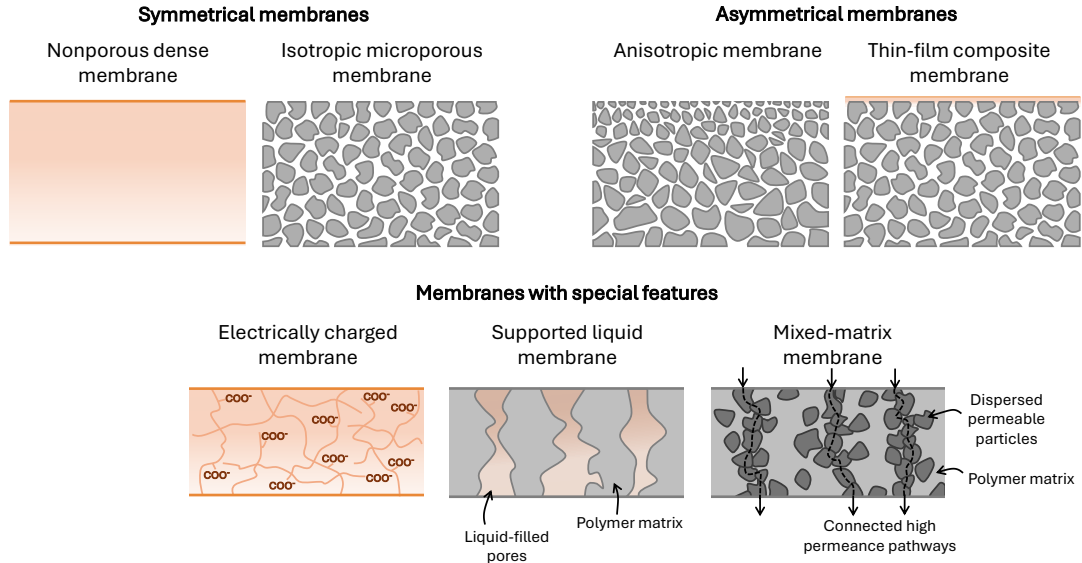


Figure 2.1: Classification of membrane structures (adapted from Baker et al. [Bake2023]).

pores occurs due to a pressure gradient. Solutes or particles smaller than the pores are retained, allowing the fluid or smaller solutes to pass. However, since most pores are not uniformly sized, perpendicular, straight cylinders, some care must be taken in quantifying pore size. The flux of a given component J_i across the membrane is described by Darcy's law:

$$J_i = K' c_i \frac{dp}{dx} \quad (2.1)$$

with the coefficient K' describing the nature of the medium, c_i as the concentration of the component i , and dp/dx as the pressure gradient across the membrane.

In the solution-diffusion model, components must first dissolve in the membrane material and then be transported across the membrane by a concentration gradient. The separation of the components occurs due to differences in solubility and diffusivity of the components in the membrane. Fick's law describes the flux of a given component J_i :

$$J_i = -D_i \frac{dc_i}{dx} \quad (2.2)$$

with D_i as the diffusion coefficient of the component i in the membrane, and dc_i/dx as the concentration gradient of component i . The negative sign indicates that diffusion occurs in the direction of decreasing concentration. Thin membranes and large concentration gradients are required to achieve useful fluxes across the membrane.

The selection of which transport model to use for which type of membrane, either dense or porous, is mainly dictated by the average pore size. However, it must be noted that pore size can often only be determined by the size of molecules retained by the membrane. Therefore, dense membranes are also described by an average pore size. While these membranes do not have an intrinsic porosity, the thermal motion of the polymer molecules enables the formation of free-volume elements that appear and disappear simultaneously to the movement of the permeating species across the membrane.[Bake2023]

Figure 2.2 showcases common membrane processes categorized by their transport mechanism and nominal pore sizes. The solution-diffusion model describes membranes with pore sizes less than 0.5 nm. An example are reverse osmosis (RO) membranes. Above 2 nm pore size, flow is described by the pore-flow model. Membranes that fall under this category are ultrafiltration (UF) and microfiltration (MF). There is no clear transition between the two domains, and membranes with pore sizes between 0.5-2 nm have properties corresponding to the two categories. An example are nanofiltration (NF) membranes [vdHors1995]. For membranes not falling in the category of dense or microporous (e.g., electrically charged membranes, liquid membranes, or mixed matrix membranes), other models have been developed to better describe the selective transport of species. These include models based on the Donnan theory [Luo2018], and different types of coupled transport and facilitated transport models [Bart1996; Vinh2013].

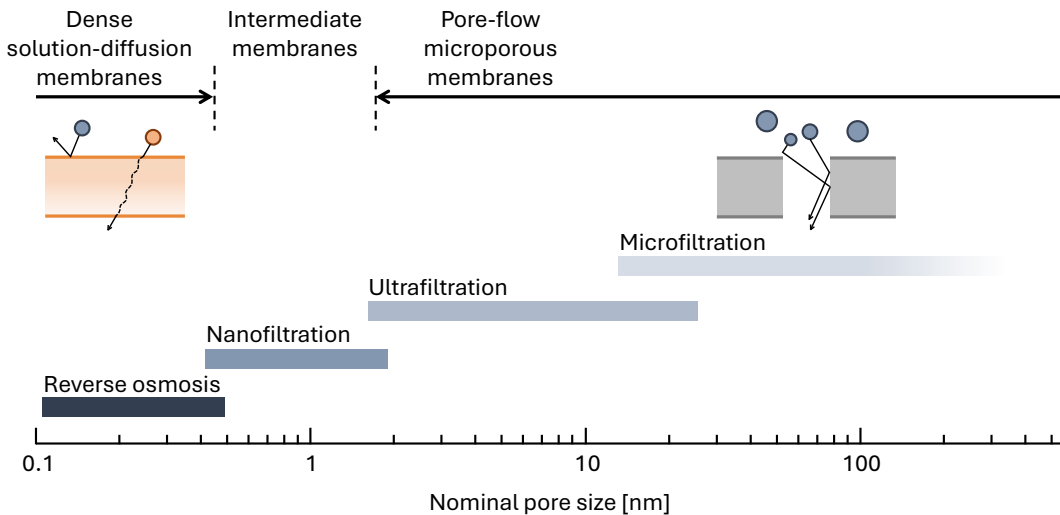


Figure 2.2: Classification of pressure-driven membrane processes according to their pore size and transport mechanism (adapted from Baker et al. [Bake2023]).

2.2 Membrane Fabrication

Synthetic membranes play an essential role in various industries, including water treatment, the medical field, and food and beverage applications. Each application has specific requirements regarding the chemical and mechanical stability of the membrane, pore size and pore size distribution, and the final configuration of the membrane module. Therefore, different materials and material processing methods have been developed over the years to achieve the specific needs of any given application.[Nune2020]

In the following, the two membrane fabrication methods more relevant for this thesis will be discussed in depth. These are non-solvent induced phase separation and aqueous phase separation.

2.2.1 Non-solvent induced phase inversion

Most of the polymeric membranes in the market today are fabricated following a phase separation process. This is accomplished by dissolving the polymer in a solvent or a mixture of suitable solvents. The polymer is usually a fossil-sourced thermoplast and the solvent is usually an organic solvent. Precipitation is achieved, for example, by immersing the polymer solution into a non-solvent coagulation bath, whereby the exchange of solvent and

non-solvent results in the solidification of the polymer. This process is called non-solvent induced phase separation, or NIPS.[Bake2023; Wien1996]

The NIPS process is simplified in the three component phase diagram in Figure 2.3. The pure components (polymer, solvent, non-solvent) are represented by the three corners of the diagram. The diagram is further divided into two main regions: a single-phase region in which any mixture of the components forms a homogeneous phase, and a two-phase region in which the polymer solution precipitates into a polymer-rich and a polymer-lean phase. The binodal curve marks its boundary. Inside the two-phase region, there is a metastable region bounded by the spinodal curve. Preparation of a membrane starts by placing a cast polymer solution (A) in a coagulation bath, initiating the exchange of solvent by the non-solvent. Consequently, the solution crosses into the two-phase region (B, B'), where further exchange of solvent leads to solidification (C). The membrane attains its final composition (D) when all solvent is exchanged. The composition of the water-swollen polymer matrix is given by S, whereas the composition of the pores consist only of water (L).

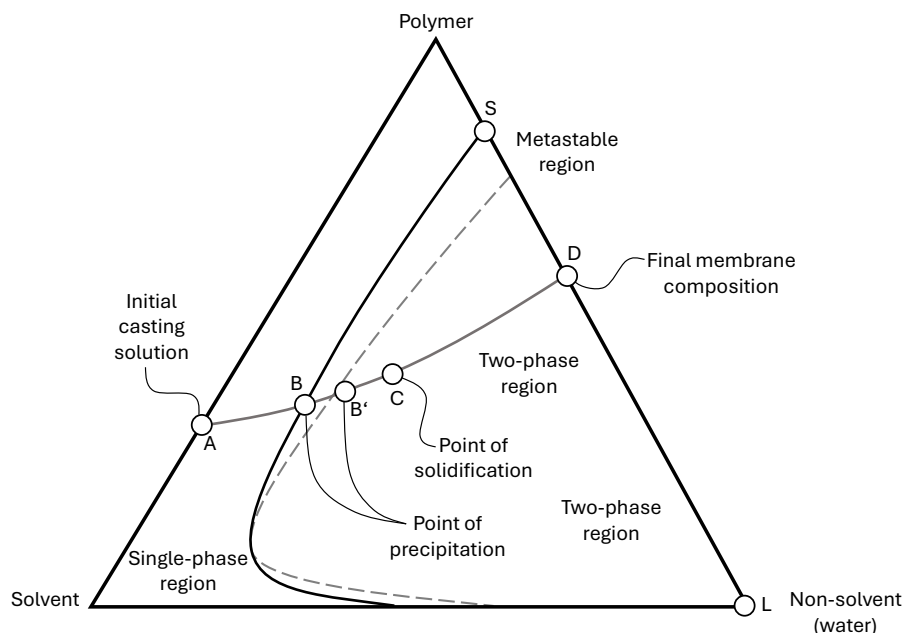


Figure 2.3: Tertiary phase diagram depicting the average composition of polymer film during non-solvent induce phase separation (adapted from Baker et al. [Bake2023]).

The diagram only displays the average composition of the membrane during precipitation. In practice, the type and rate of precipitation vary over the film depth, leading to variations in pore structure across the membrane cross-section. For instance, precipitation at the outer layer occurs more rapidly due to the unhindered exchange of solvent and non-solvent. The resulting structure is usually relatively dense and finely microporous since the newly formed phases do not have time to segregate [Broe1980]. Moreover, the formation of a dense top layer further hinders the solvent exchange in the layers beneath. Here, phase separation occurs more slowly, allowing for the growth of two distinct phases before the polymer is solidified. Hence, pore size gradually increases with increasing film depth [Bake2023]. Nonetheless, other factors such as the type of polymer, solvent and non-solvent, polymer solution viscosity, casting conditions, and coagulation bath composition and temperature can also significantly affect the final membrane morphology [Guil2011].

Over the past decades, extensive knowledge has been gained on the influence of fabrication parameters on the resulting membrane structure and performance. This way, membranes with pore sizes ranging from tight UF to open MF can be fabricated using the same solvent-polymer system. Unfortunately, membrane fabrication via NIPS comes with a clear downside: commercial fabrication of membranes via NIPS involves the use of fossil-sourced, environmentally harmful, and repro-toxic polar aprotic solvents [Figo2014; Zou2021]. For this reason, commonly used solvents such as *N*-methyl-pyrrolidone (NMP) and *N,N*-dimethylformamide (DMF) have come under intense scrutiny and are currently restricted by the EU under the REACH accord [Bour2016]. Therefore, there are great incentives to replace commonly used toxic solvents with greener, more sustainable alternatives [Jord2022; Figo2014].

2.2.2 Aqueous phase separation

In recent years, aqueous phase separation (APS) developed as an alternative to NIPS for membrane fabrication (see Section 2.2.1. APS uti-

lizes water-soluble polyelectrolytes and their interactions in aqueous environments to form free-standing polyelectrolyte (PE) membranes. PEs are charged polymers with positive or negative charges on their repeating units. To ensure electroneutrality, PEs are accompanied by small counterions that stabilize the charge of the repeating units. When oppositely charged PEs mix in water, the entropy gain from the counterion release leads to the formation of solid-like aggregates, commonly known as polyelectrolyte complex (PEC) [Mich1965].

The knowledge of PEC formation has triggered a wide range of applications in various fields. PECs can form structures with well-defined geometry for applications such as encapsulation and drug delivery [Voet2009], be processed into plastic-like materials called saloplastics with excellent material properties [Scha2015], or be assembled into thin PE multilayers. The latter was introduced by Decher et al. in 1991 and involves the alternate deposition of polyanions and polycations, now commonly known as the layer-by-layer (LBL) technique [Dech1997; Dech1991]. LBL has gained widespread attention in membrane fabrication as it allows the precise tuning of membrane properties, enabling the fabrication of highly selective membranes for NF, RO, and gas separation [Cho2014; Jin2003; Leva1995]. Though powerful, this layer-by-layer technique is time-consuming and requires a support membrane fabricated through typical phase inversion techniques such as NIPS.

More recently, knowledge of PEC formation has also expanded into the fabrication of free-standing PEC membranes. This development arose from understanding the phase behavior of PEs and PECs in aqueous environments under specific conditions, as shown in Figure 2.4. As previously mentioned, when PEs with opposite charges are combined in an aqueous environment, they interact and form solid-like aggregates (left). For strong polyelectrolytes (whose charge is independent of surrounding pH), this interaction can be hindered by increasing the ionic strength of the solution through salt addition [Wang2014]. For weak polyelectrolytes (whose charge depends on the surrounding solution), interaction can be prevented by adjusting the solution's pH to render the polyelectrolyte uncharged [Baig2020].

Depending on salt concentration, pH, or temperature, coacervation of the solid and the surrounding liquid phase can be initiated (middle). Coacervation describes the liquid-liquid phase separation in aqueous solutions of oppositely charged macromolecules. In this state, the polymer lean phase is in equilibrium with the polymer-rich phase. The coacervate can transform into a homogeneous liquid polymer solution by further increasing the ionic strength of the solution or by adjusting the pH (right). In this state, no interactions between the PEs are present.

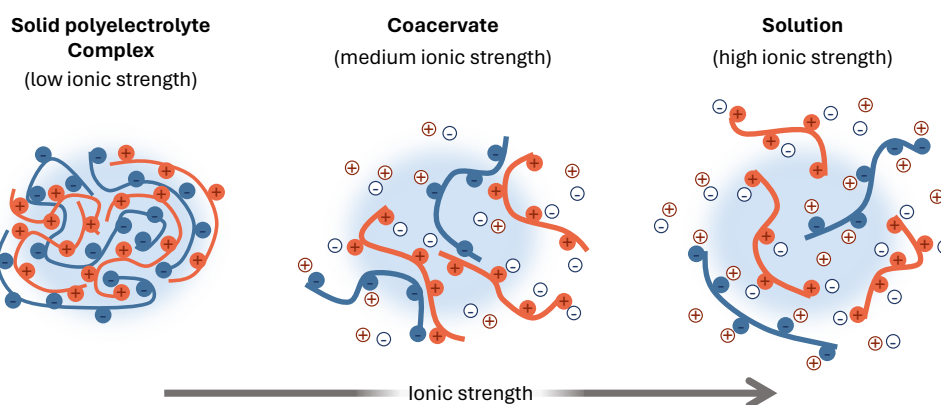


Figure 2.4: Phase behavior of oppositely charged polyelectrolytes at different ionic strengths (adapted from Wang et al. [Wang2014]).

Thus, solutions of oppositely charged polyelectrolytes can be mixed into a homogeneous solution, and porous structures can be obtained through controlled precipitation of this solution. Precipitation is achieved by removing excess salt ions (low salinity coagulation bath) or by shifting the pH. This method has been used to fabricate porous polyelectrolyte complex particles and membranes with varying properties [Baig2021; Kamp2021a; Durm2020; Rest2024; Wang2015]. APS closely mirrors the previously discussed NIPS, as it involves the replacement of a solvent, which in this case can be considered as a salt, an acid, or a base, by a non-solvent, which is water. Therefore, the rate at which the solvent and the non-solvent are exchanged greatly influences the morphology and the performance of the resulting membranes. Indeed, membranes with properties ranging from NF, UF, MF, as well as gas separation and ion-exchange membranes have been fabricated [Baig2021; Kamp2021a; Durm2020; Rest2024; Li2021; B2021].

2.2.3 Dry-jet wet hollow fiber spinning

The simplest method for membrane fabrication via phase inversion is flat sheet (FS) casting. A thin film is cast on a suitable support and immersed in a coagulation bath. While free-standing membranes can be fabricated by casting the polymer solution directly on a glass or metallic plate, industrial processes usually cast membranes on a moving, non-woven support [Bake2023]. The support facilitates fabrication and ensures mechanical stability. However, this thesis predominantly discusses hollow fiber (HF) membranes. However, this thesis predominantly discusses the use of hollow fiber (HF) membranes. HFs are cylindrically shaped membranes with the advantage of providing a higher surface area per module volume than FS. Moreover, HFs are self-supported and can withstand high pressures at comparatively low wall thickness [Peng2012].

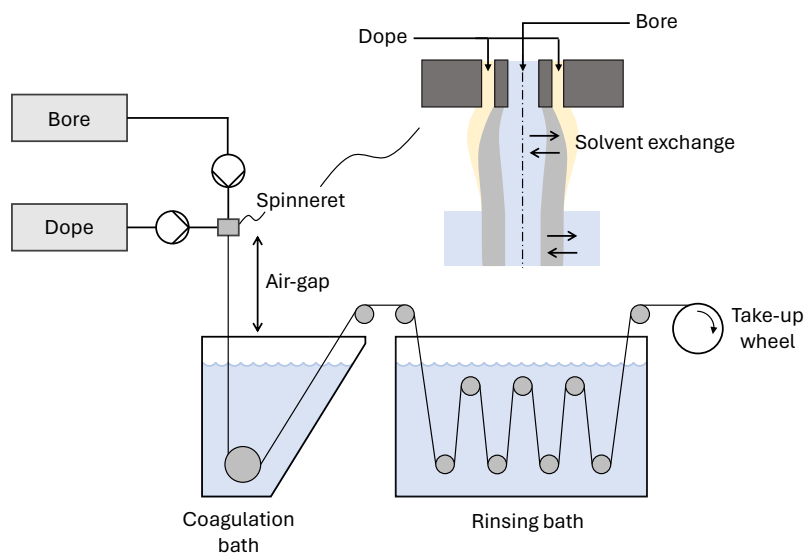


Figure 2.5: Schematic representation of a hollow fiber spinning process.

HF via NIPS is done by a dry-jet wet spinning process as showcased in Figure 2.5. The heart of the spinning process is the spinneret, which, in its simplest form, consists of two concentric capillaries (upper right). The polymer solution is extruded through the outer capillary, while a bore solution is forced through the inner one. The bore solution prevents the HFs from

collapsing and usually consists of a mixture of solvent and non-solvent. The fiber is first extruded into an adjustable air-gap, at which point precipitation already begins thanks to the solvent and non-solvent exchange in the lumen of the fiber. The nascent fiber then falls into a non-solvent coagulation bath where further solvent exchange occurs, and the fiber solidifies. Finally, the fibers are collected by the take-up wheel which at the same time exerts tension to the fibers and dictates the conveying speed.[Peng2012]

During HF spinning, parameters such as the composition and temperature of the polymer and bore solution, extrusion velocity of the polymer and bore solutions, air-gap length, composition and temperature of the coagulation bath, air humidity, take-up speed, and spinneret geometry can be varied. Due to this complexity, there have been efforts to summarize the effects of the various parameters on the resulting membrane properties [McKe1997].

2.3 Membrane functionalization

Most polymeric membranes in the market today are made from a small selection of polymers. These include various thermoplastic materials such as polysulfone, polyethersulfone, polyvinylidene fluoride, or cellulose acetate [Bake2023]. While these materials offer a good variability of pore sizes and mechanical and chemical stability, some applications require specific characteristics not given by the base polymer.

2.3.1 Post-modification

Membrane post-modification is one of the most researched topics in membrane science. Over the years, a wide variety of methods for imparting membranes with desired properties have been developed. Post-modification consists in the immobilization of one or more layers on the membrane surface via covalent attachment by adsorption via ionic or hydrophobic interactions. Some of the most widely researched post-functionalization methods are interfacial polymerization, LBL coating,

plasma treatment, and grafting [Brug2009].

Historically, one of the most important post-modification methods is interfacial polymerization (IP). IP consists of the reaction of at least two monomers at the interface of two immiscible phases. In this process, the support membrane is soaked with an aqueous solution containing a functional amine, e.g., m-phenylenediamine (MPD). The support is then brought into contact with an organic solution containing a functional acyl chloride such as trimesoyl chloride (TMC). At the interface between the non-miscible aqueous and organic phases, the two monomers react to form a thin and dense selective polyamide (PA) layer. The thin PA layer retains most molecules besides water, so PA-TFC membranes remain the most efficient method for water desalination.[Cado1980]

However, one of the main drawbacks of PA TFC membranes is their low chemical stability against chlorine [Kang2007]. More recently, the commercial fabrication of chlorine-stable NF membranes was made possible by large-scale LBL PE coating [Jonk2023]. LBL consists of the alternate adsorption of oppositely charged polyelectrolytes on the membrane surface, allowing for the precise tailoring of the membrane properties regarding ion selectivity and permeability (see Section 2.2.2). Polyelectrolyte adsorption has also shown beneficial for decreasing fouling in membranes [Ishi2012], which is especially interesting for applications in the food and beverage industry and water treatment [Moha2012]. Furthermore, some polyelectrolytes also allow for the subsequent cross-linking of functional molecules such as proteins and enzymes [Dizg2018; Li2018; Li2020].

In plasma modification, membranes are subjected to a plasma atmosphere generated by the ionization of gas [Brug2009]. In this process, only the top layer of the membrane surface is altered, meaning that the bulk composition of the polymer remains unchanged. Depending on the type of gas used, different kinds of functional groups can be introduced to the membrane surface. For example, nitrogen-based gases introduce amine functionalities (e.g. -NH₂, -NH, etc.), while oxygen-based gases introduce oxygen-containing functional groups (e.g., -OH, -C=O, -COOH) [Brug2009]. Moreover, if inert gases such as argon or helium are used, free radicals

are generated, which can then react with other monomers to bind these to the surface covalently. This process is also commonly known as grafting [Bhat2004].

In general, grafting refers to the process of covalently attaching molecules such as monomers or polymer chains to the surface of a material [Bhat2004]. This technique is widely applied in membrane technology to enhance hydrophilicity, often by incorporating highly hydrophilic polymer brushes such as PEG chains or polyelectrolytes [Rana2010]. Grafting can be achieved through various methods, for example, plasma treatment, UV irradiation, or exposure to radiation [Brug2009; Rana2010].

2.3.2 Blending

One of the simplest methods for membrane functionalization is blending. Blending involves the addition of functional materials in the polymer solution prior to membrane formation. In contrast to other post-modification techniques, this process is less time-consuming and does not involve special coating/modification apparatus. However, the additives must be soluble in the solvent and compatible with the polymer-solvent system.

Materials used for blending can range from nanoparticles and inorganic fillers, to different polymers and co-polymers. Nanoparticles can be used to improve the permeability of membranes, decrease fouling, and impart the membrane with catalytic properties [Ng2013]. Commonly used nanoparticles are silver, titanium, and iron-based nanoparticles, among others [Ng2013]. Larger inorganic fillers, such as metal-organic frameworks, zeolites, and silica particles, can be used to fabricate mixed matrix membranes (MMM). The role of inorganic fillers in MMM is most largely used for improving the permeance and selectivity of gas separation membranes [Dech2017]. The last type of additives are polymeric materials. In conventional membrane fabrication via NIPS, water-soluble polymers such as polyvinylpyrrolidone (PVP) and PEG have been used to enhance the porosity and control the pore size of membranes [Guil2011]. Moreover, thanks to their hydrophilic character, these polymers can improve mem-

brane hydrophilicity and reduce fouling [Wang2013]. However, their stability during operation is limited as these hydrophilic polymers also easily wash away [Miya1990]. The use of amphiphilic co-polymers has been studied to prevent leaching. In this case, the hydrophilic segments of amphiphilic co-polymers separate towards the membrane surface due to their low interfacial energy with the water coagulation bath. Simultaneously, hydrophobic segments securely attach within the membrane, facilitated by their hydrophobic interactions with the polymer matrix [Yi2024].

2.4 Enzymatically active membranes

Enzymes are the powerful catalysts of living organisms; almost all cell metabolic processes need enzyme catalysis to occur at rates fast enough to sustain life. Enzymes, which in most cases are proteins (with the exception being ribozyme), are characterized by high specificity upon the molecules they act on, all while operating under mild conditions (physiological pH and temperature). Moreover, since enzymes are biodegradable, non-toxic, and non-hazardous, there is no risk of product and environmental contamination from catalyst residues [Shel2021]. These characteristics enable the use of enzymes in a variety of applications ranging from very delicate, such as the inactivation of toxins in human blood [Labe1993; Pete2014], to more industry relevant, such as the removal of CO₂ from exhaust gases [Moli2021].

However, to date industrial use of enzymes is mostly limited to the pharmaceutical and the food and beverage industry, as well as for fabric and household cleaning [Cosi2013]. This is due to several reasons: first, enzymes are highly sensitive to process conditions and are easily damaged under high temperatures, non-optimal pH, or organic solvents. Secondly, enzymes can be expensive to produce, and their difficult recovery makes processes involving soluble enzymes costly. Third, enzyme applications compete with well-established chemical processes optimized for decades [Otte2005]. Nonetheless, the need for more sustainable processes has again emphasized using enzymes as an environmentally friendly alternative to conventional catalysts [Shel2021].

One approach to ensure the commercial viability of enzymatic processes is process intensification. This can be achieved, for example, by coupling the enzymatic reaction to the product extraction and enzyme recovery through enzymatic membrane reactors [Joch2011; Mazz2021]. Indeed, enzymatic membrane reactors have emerged as a promising technology for the efficient and sustainable production of bulk and specialty chemicals, removing pollutants from air and wastewater, or even as possible treatments in the medical field [Urag2013; Luo2020; Pekg2022; Gior2000; Barb2022; Mazz2021]. Nonetheless, enzymes in membrane reactors may still be

damaged if operated under non-optimal conditions. Therefore, several approaches may be used to improve their stability. For instance, the structure of the enzymes can be engineered via directed evolution to sustain harsher environmental conditions [Sen2007]. Moreover, enzymes can be immobilized on a support material (in this case, the membrane), which has repeatedly been shown to increase the stability of the biocatalyst [Garc2011]. In membranes, enzymes can be immobilized inside the porous structure or on the selective layer [Luo2020]. However, commercially available support membranes usually lack the appropriate chemistry of the material to immobilize enzymes; therefore, surface modification is necessary [Joch2011]. Section 2.3 gives an overview of different functionalization approaches. A summary of the different enzyme immobilization methods is given in the following.

2.4.1 Enzyme immobilization

While enzymes, as a type of biocatalysts, exhibit many favorable properties like high selectivity, chemical activity at mild conditions, and biodegradability, their two main drawbacks are insufficient stability and difficult recovery from the reaction broth for reuse. Immobilizing enzymes on surfaces, particles, microcapsules, or aggregates can significantly simplify recovery. Additionally, the stability towards harsh pH values, temperature, and organic solvents is often improved due to the rigidification of the enzyme [Guis2020; Dwev2016]. However, rigidification and conformational change can lead to a change in kinetic properties and a decrease in intrinsic activity. Mass transfer limitations can lower efficiency, especially for large or poorly soluble substrates. Since the enzyme is immobilised in a microenvironment separated from the bulk solution, local charge distribution and mass transfer limitations can lead to so-called partitioning effects [Kalt1982]. For example, a positive surface charge of the used immobilization matrix results in the displacement of H^+ -ions, creating an alkaline microenvironment [Guis2013].

The concept of attaching enzyme to a fixed support instead of using it in solution was first described by Nelson and Griffin in 1916 [Nels1916] for

invertase adsorbed on charcoal. Today, various immobilization methods are available, based on both physical and chemical interaction between enzyme and support material [Guis2013]. Methods like microencapsulation [Dwev2016], cross-linked enzyme crystals [Quio1964] and cross-linked enzyme aggregates [Shel2007] aim at producing enzyme particles that can be removed easily from a solution. Since they cannot be applied for enzyme immobilization on an existing surface, they are not further discussed in this chapter. Approaches leading to enzymes immobilized on a solid support material are described in the following section.

Adsorption

Enzyme immobilization by adsorption is the oldest and, in most cases, the simplest method. It is based on non-covalent linkages like hydrophobic interaction, van der Waals force, hydrogen bonds, or electrostatic interactions between enzyme and support [Dwev2016; Datt2013]. Various support materials can be used, and in most cases, a preactivation of the support is not necessary. Due to the very mild treatment, configurational changes are rare. On the downside, intensive optimization is necessary for a durable immobilization due to the reversible character of the linkages and hence high susceptibility towards temperature, pH, ionic strength, and used solvent [Dwev2016; Datt2013]. To counteract this, a special type of adsorption can be used, the so-called affinity immobilization. Affinity immobilization exploits specific biospecific interactions, such as enzyme-cofactor or enzyme-inhibitor binding, to enhance binding stability while maintaining mild attachment conditions [Guis2013]. This approach can improve enzyme retention and activity, reducing desorption under operational conditions [Moha2015]. However, specific modifications must be conducted to enable these interactions, such as the prior immobilization of the enzyme co-factors, antibodies, or specific metal chelates [Moha2015].

Covalent attachment

While adsorption relies on non-covalent linkages, covalent bonds can also be used to immobilize enzymes. A variety of different coupling reactions between support and enzyme are known. These rely on the interaction between functional groups in the enzyme and the support, usually amino, carboxyl, and thiol groups [Moha2015]. In most cases, the support may still require pre-activation with a suitable cross-linker to enable the attachment of the functional groups. Cross-linkers are highly reactive species, typically aldehydes or epoxides, that facilitate covalent bonding by forming stable linkages between the enzyme and the support material. However, it is also possible to form covalent bonds between support and enzyme without cross-linkers. In this case, the support material in the presence of the enzyme (e.g., immersed in a buffer solution containing the enzyme) is activated by an electron beam (EB). The irradiation with the EB leads to the formation of radicals in both the support material and the enzyme, which under optimal conditions recombine to form covalent bonds between enzyme and support [Star2013]. This method does not require any prior surface functionalization or the use of toxic cross-linkers [Star2013]. However, it still requires the use of specialized equipment.

Covalently attached enzymes are usually irreversibly bound to the support [Guis2013], resulting in increased stability towards physical and chemical stress [Dwev2016]. Especially when more than one bond between enzyme and support is formed, the strain on the enzyme can introduce significant conformational changes and consequently loss in activity [Dwev2016]. A pre-treatment with a cross-linker like glutaraldehyde (GA) can rigidify the enzyme's structure, especially in multimeric enzymes [Datt2013]. Steric hindrance due to proximity to the support can be avoided by introducing a spacer during the immobilization process [Dwev2016].

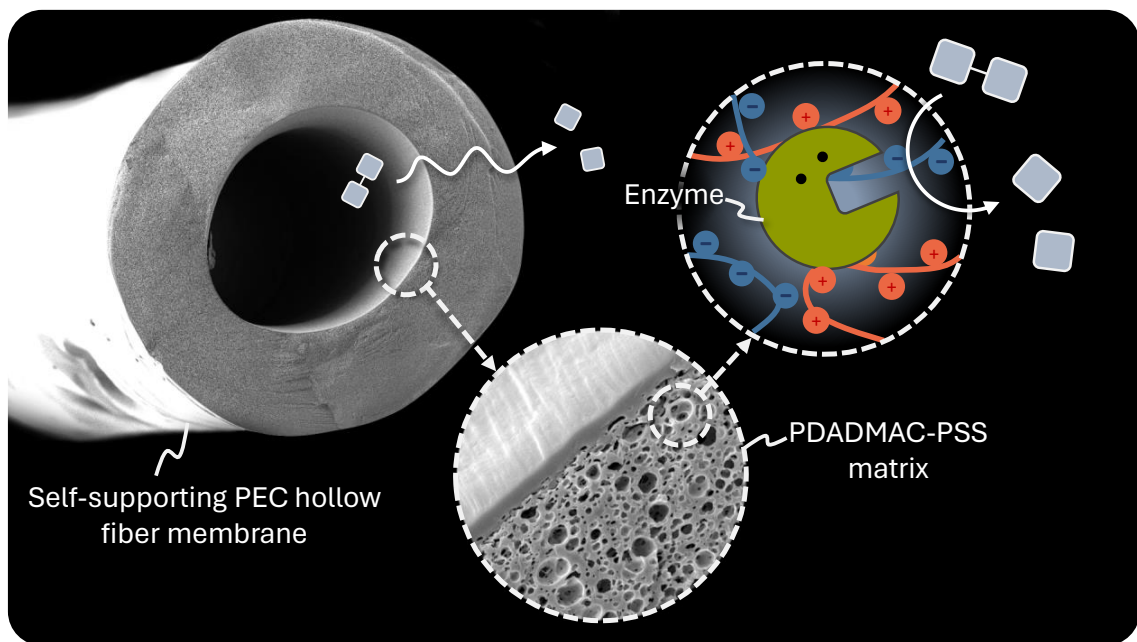
Entrapment

The third common option for enzyme immobilization on a solid support is entrapment. Here, the enzyme is physically captured in a polymer

matrix [Dwev2016]. The matrix can be formed by covalent and non-covalent bonds [Datt2013]; common materials are polyacrylamide and alginate [Das1998]. The matrix is designed to retain the enzyme while allowing ideally unhindered diffusion of substrate and product to the active site. Since this clear cut-off cannot be ensured completely, enzyme leakage is a common problem for this technique. As an advantage, entrapment usually does not introduce any intrinsic change to the enzyme, hence retaining enzyme activity very well [Dwev2016]. On the downside, mass transfer to and from the enzyme is often reduced [Rome2019].

3

Biocatalytic polyelectrolyte complex hollow fiber membranes



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3.1 Introduction

Polyelectrolytes (PE) offer fascinating opportunities for membrane modification and fabrication. Due to their ionic nature, most PEs are readily soluble in water, avoiding the need of organic solvents for their processing. Indeed, thanks to their versatile properties, PEs are used in various fields, e.g. pharmaceutical and biomedical, water treatment, cosmetics, and food and paper industries. Especially in the pharmaceutical field, PEs are widely researched for their use as biocompatible and controllable coating materials. [Durm2020; Boud2010; Lung2005; Meka2017; Schm2011; Kuma2007; Mort1991]

When PEs with opposite charges are present in an aqueous environment, ionic interactions result in complexation in the form of solid aggregates [Mich1965]. The polyelectrolyte complexation process can be suppressed if the interaction between ionic groups is prevented (see Section 2.2.2). For strong PEs (the charge is independent of the pH of the surrounding solution), the presence of sufficiently high salt concentrations shields the charges of PEs [Wang2014]. Eventually, phase separation occurs with the formation of a coacervate phase. Above an overcritical salt concentration, the ionic interactions between PEs are negligible and they completely dissolve in a homogeneous polymer solution [Wang2014].

Enzymes can be categorized as weak PEs with both positive and negative charges on their surface, with the protein's net charge being dependent on the pH of the surrounding solution. Therefore, enzymes participate in phase separation by forming complexes with charged polymers or with two oppositely charged PE [Horn2019; Blac2014; Kayi2013]. Enzyme-polyelectrolyte complexes have been previously reported to enhance enzymatic activity and stability. The beneficial electrostatic interactions between enzymes and complementary charged PEs allow the enzyme to retain its native structure, improving thermostability, resistance to non-conventional media and storage stability [Thie2018; Gang2009; Kaca1994; Tang2022; Tira2013].

Protein and enzyme immobilization using polyelectrolyte complexes has

been extensively researched over the years. This includes the encapsulation of enzymes in coacervates with possible applications in drug delivery, as nanoreactors, protocells, and membrane-less organelles [Bloc2017]. For instance, surface functionalization may be achieved through subsequent deposition of polyelectrolyte layers through the layer-by-layer (LBL) method (see Section 2.2.2). Enzymes can be immobilized inbetween or on the top layers through entrapment, adsorption, or covalently using cross-linkers [Sakr2013; Sigu2018]. However, LBL remains a time-consuming process due to the multiple coating and washing steps. More recently, Lim et al. described the single-step immobilization of the enzyme glucose oxidase via a salt-dilution induced phase separation from a liquid coacervate to a microporous, solid material [Lim2020].

One advantage of using PECs as a matrix for enzyme immobilization is that PECs can be processed to selective microporous membranes. Various groups recently demonstrated successful membrane fabrication through controlled precipitation of a homogeneous solution containing oppositely charged PEs [Sadm2019; Kamp2021a; Durm2020; Baig2020; Baig2021; Miza2023]. Currently two approaches are described based on the types of PEs used. For example, if salt is used to dissolve PEs, then immersion of the polymer solution in a low salinity coagulation bath will lead to phase separation, a process also described as salt-dilution-induced phase separation (SIPS) [Sadm2019; Kamp2021a; Durm2021]. On the other hand, if one of the PEs becomes ionized only at a given pH range, immersing the polymer solution in a coagulation bath with that given pH will lead to phase separation (pH-shift induced phase separation) [Baig2020; Baig2021]. Both coagulation processes occur analog to the non-solvent induced phase separation (NIPS). Hence, it is demonstrated that adjusting the characteristics of both polymer solution and coagulation bath leads to a wide range of membrane morphologies and separation characteristics. Moreover, since no organic solvents are involved in the fabrication of PEC-based membranes, enzymes may be directly incorporated during the fabrication process.

Recently, van Lente et al. produced biocatalytic membranes in a single step through the precipitation of a homogeneous polymer solution contain-

ing an enzyme, polyanions, and polycations via pH-shift [vLent2022]. Nevertheless, their work was limited to flat sheet membranes. Hollow fibers, on the other hand, allow for a higher surface-to-volume ratio which results in a larger biocatalytic area per module volume. To date, only two publications describe the fabrication of hollow fiber and tubular PEC membranes [Baig2022; Emon2021]. However, both raise the issue of insufficient mechanical strength. Baig et al. used glutaraldehyde to enhance the mechanical strength. Still, the resulting micro and ultrafiltration membranes were measured only up to 1 bar transmembrane pressure (TMP) in an outside-in configuration. Moreover, Emonds et al. produced inside-out tubular membranes with nanofiltration properties, but a ceramic support was required to withstand the applied TMP of 3 bar.

This work explores the immobilization of the enzyme alkaline phosphatase (ALP). ALP is an enzyme responsible for the hydrolysis of extracellular organic phosphate esters. The immobilization of ALP on various substrates has been extensively researched, as ALP is known to promote bone formation in tissue engineering [Oort2012; Osat2009]. However, ALP has also been shown to be effective in reducing the mortality rate in patients suffering from acute kidney injury related to sepsis. During sepsis, ALP can reduce inflammation through the phosphorylation of endotoxins and the inflammatory mediator adenosine triphosphate. However, ALP is rapidly metabolized by the body and quickly loses its therapeutic effect [Pete2014]. Therefore, the immobilization of ALP in a blood treatment device is of medical importance, even more so in combination with a membrane that further ensures the removal of waste molecules from the blood stream [Swar2022]. Indeed, the use of biocatalytic membranes in the medical field is feasible because of the short life span of the devices and the mild reaction conditions.

This chapter discusses a direct immobilization method for ALP through the complexation of poly(sodium 4-styrenesulfonate) (PSS) and poly(diallyl dimethylammonium chloride) (PDADMAC), as illustrated in Figure 3.1. By taking advantage of the favorable interactions between the PE chains and charged groups on the enzyme surface, the enzyme stability during operation is enhanced, and enzyme leaching is reduced.

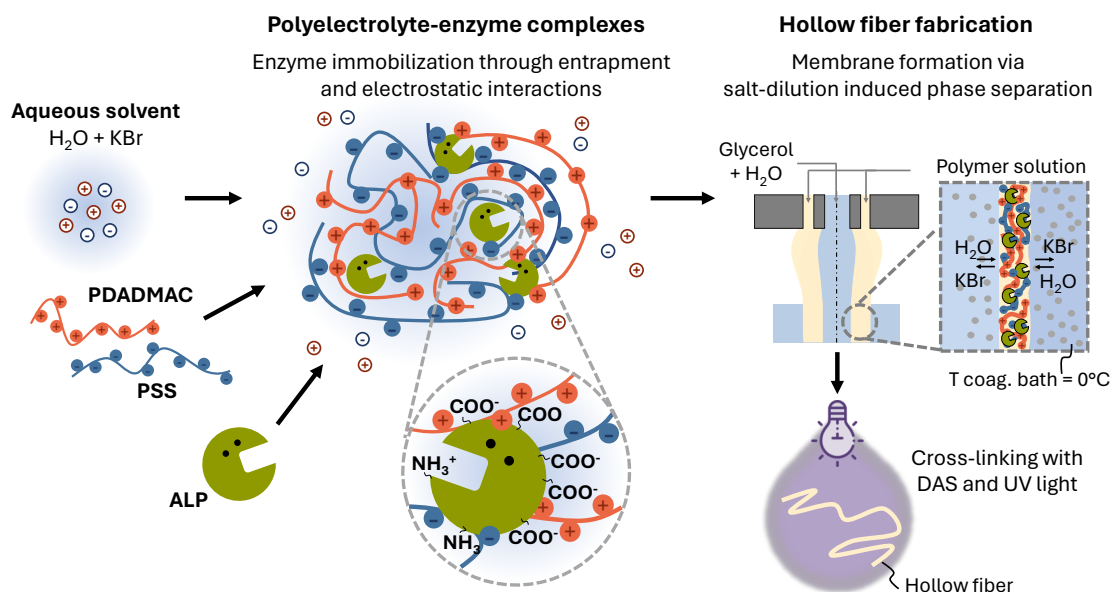


Figure 3.1: Enzyme immobilization and hollow fiber membrane formation through the complexation of oppositely charged polyelectrolytes from an homogeneous polymer solution via the salt-dilution induced phase separation (SIPS).

This simple yet effective immobilization technique can be applied not only in the formation of free-standing porous ALP-PEC films but also in more complex hollow fiber membranes. However, realizing this ambition presents challenges that need to be addressed. Firstly, the optimal spinning conditions for producing defect-free and mechanically stable hollow fiber membranes must be identified. Secondly, a post-processing method must be developed to stabilize the hollow fiber membranes through cross-linking while preserving the enzyme's reactivity.

To tackle these challenges, a cross-linking method is proposed using 4,4'-diazidostilbene-2,2'-difulfonate tetrahydrate (DAS), a UV photoreactive cross-linker. This approach demonstrates the potential of this novel manufacturing process not only as a green alternative to standard organic solvent-based approaches but also as a versatile platform for the generation of biocatalytic membrane materials.

3.2 Materials and Methods

3.2.1 Materials

Poly(sodium 4-styrenesulfonate) (PSS) with high molecular weight (MW) (average MW $\sim 1,000,000$ powder) and poly(diallyl dimethyl ammonium chloride) (PDADMAC) with high MW (average MW $\sim 400,000$ - $500,000$; 20 wt% in H_2O) are supplied from Sigma Aldrich. Potassium bromide (KBr) (99 %) was purchased from Alfa Aesar. The cross-linker disodium 4,4'-diazidostilbene-2,2'-difulfonate tetrahydrate (DAS) $>98\%$ was obtained from TCL. Alkaline phosphatase (ALP) from bovine intestinal mucosa (lyophilized powder, ≥ 10 U/mg), p-nitrophenol (pNP) (10mM solution), 2-Amino-2-methyl-1-propanol (AMP) ($\geq 95\%$), Tris-Buffered saline solution (TBS), sodium hydroxide (NaOH) ($\geq 98\%$) are supplied by Sigma Aldrich. For the enzyme activity measurements, a ready-to-use p-nitrophenylphosphate solution (pNPP) was purchased from Sigma Aldrich. For ALP labeling, NHS fluorescein (5/6-carboxyfluorescein succinimidyl ester) and dimethylsulfoxide (DMSO) $>99.9\%$ were purchased from Thermo Fisher. For molecular weight cut-off measurements, polyethylene glycols (PEG) with different sizes (200, 400, 600, 1000, 1500, 4000, and 6000 g mol^{-1}) were supplied by Sigma Aldrich.

3.2.2 Membrane fabrication

Flat sheet and hollow fiber membranes were prepared using a polymer solution with an equimolar monomer ratio of 1:1 PSS to PDADMAC. This ratio was calculated using the molecular weight of the monomers $M_{PSS}=205.6\text{ g mol}^{-1}$ and $M_{PDADMAC}=161.67\text{ g mol}^{-1}$. The salt concentration used, calculated as the ratio of KBr to water, was $2\text{ mol}_{KBr}/\text{kg}_{H_2O}$. For polymer solutions containing ALP or fluorescent labeled ALP (fALP), a concentration of $0.1\text{ mg}_{ALP}/\text{g}_{\text{solution}}$ was used. The detailed composition of the solution is presented in Table 3.1. First, PSS, PDADMAC, and KBr were mixed under vacuum using an asymmetric centrifugal mixer (SpeedMixer, Hauschild, Germany) at 1750 rpm until PSS was completely dissolved. ALP

and DI-water were vortexed separately for 10 s and then added to the polymer solution. The polymer solution was again mixed under vacuum until a homogeneous solution was obtained.

Table 3.1: Composition of the polyelectrolyte polymer solutions for the flat sheet and hollow fiber membrane fabrication.

	Solution -	PDADMAC:PSS monomer ratio	PSS [wt.%]	PDADMAC [wt.%]	KBr [wt.%]	H ₂ O [wt.%]	Enzyme [wt.%]
Flat sheet	S.1	1:1	11.21	8.79	15.36	64.54	0.1 (ALP)
	S.2	1:1	11.21	8.79	15.38	64.52	-
Hollow fiber	S.3	1:1	14.01	11.01	15.06	60.12	0.1 (ALP)
	S.4	1:1	14.01	11.01	15.06	60.12	0.1 (fALP)
	S.5	1:1	14.03	11.01	15.03	60.07	-

For the fabrication of PEC films, 200 μm thick films were cast on an aluminum plate with a casting knife. Subsequently, the films were submerged in DI-Water, causing the KBr to diffuse out of the film, initiating the PE complexation. The coagulated films were stored in DI-water for at least 1 h to allow the salt to diffuse out of the films. The films were then transferred to fresh deionized (DI) water and stored at 4 °C until further use.

For hollow fiber membrane fabrication, the prepared homogeneous polymer solution was extruded with a bore fluid through a spinneret with a single orifice. The bore needle has an inner diameter of 0.8 mm and an outer diameter of 1.5 mm. The fiber was immersed in an aqueous coagulation bath at 0 °C which initiates precipitation. Then, the hollow PEC fibers were drawn into a second water bath with a pulling wheel, where they were allowed to collect, ensuring complete precipitation. The hollow fiber membranes were immersed in a 50 wt % glycerol/water solution for 24 h. Subsequently, the fibers were stored in DI water at room temperature until further use.

Table 3.2: Spinning parameters for the hollow fiber membrane fabrication.

	Solution -	Temp. [°C]	Air-gap [cm]	Dope flow [mL/min]	Roll speed [m/s]	Bore [kg/kg]	Bore flow [mL/min]
HF.ALp	S.3	0	1.5	0.5	0.075	1:1 Glycerol/H ₂ O	0.1
HF.fALP	S.4	0	1.5	0.5	0.075	1:1 Glycerol/H ₂ O	0.1
HF.ref	S.5	0	1.5	0.5	0.077	1:1 Glycerol/H ₂ O	0.1

Hollow fiber membrane post-treatment and cross-linking

To improve the mechanical stability of PEC membranes, a photo-induced cross-linking reaction is applied. First, the hollow fiber membranes were immersed in a 10 g L^{-1} cross-linker (DAS) solution to allow the cross-linker to diffuse into the membrane structure. Afterwards, the prepared membranes were placed in a transparent UV-stable acrylic tube and then irradiated with UV light at a wavelength of 350 nm for 10 min. To achieve homogeneous irradiation, three LED arrays were equally distributed around and across the total length of the tube. Finally, cross-linked membranes were collected and stored in a 50 wt % glycerol/water solution.

3.2.3 Light transmittance measurements

To measure the precipitation velocity of the polymer solution used for hollow fiber fabrication, films of 200 μm thickness using the same polymer solution as the hollow fibers (S.5, see Table 3.1) were cast on a glass plate and immersed in a light transmittance measuring cell. A detector placed behind the glass plate captured the intensity of light emitted from a light source placed before the glass plate.

3.2.4 Enzyme fluorescein labeling

NHS-fluorescein labeled ALP (fALP) was prepared by solving 50 mg mL^{-1} of ALP in 1 mL PBS. Furthermore, a 300 μL solution of 10 mg mL^{-1} NHS-fluorescein in DMSO was prepared. Subsequently, 1 mL of ALP in PBS was mixed in a reaction tube with 266 μL of NHS-fluorescein in DMSO. The tube was incubated for 2 h at $4\text{ }^{\circ}\text{C}$. Afterwards, the labeled enzyme was purified with a Merck Amicon® Ultra 0.5 ml centrifugal filter with a MWCO of 30 kDa. Samples were centrifuged at 13 000 rpm. After 2 h, the samples were rinsed with 200 μL of PBS and subsequently centrifuged again for 30 min. This rinsing step was repeated three times in total for each tube. Finally, the supernatant was removed and the fluorescein-labeled ALP was redispersed two times with 250 μL PBS. The PBS with enzyme was collected, resulting

in a total of 1.5 mL with a maximum of 30 mg mL⁻¹ fALP.

3.2.5 Field emission scanning electron microscopy

To analyze the morphology of the membranes, FESEM imaging was used. Cross-sectional images were prepared by fracturing the samples in liquid nitrogen to obtain sharp edges. Then, the samples were freeze dried and were subsequently stored in an desiccator for one week. FESEM imaging was performed using a Hitachi SU5000.

3.2.6 Optical analysis by fluorescence microscopy

For the visualization of the surface of ALP-PEC hollow fibers, confocal laser scanning microscopy with a TCS SP8 FALCON (Leica, Germany) was used. To obtain cross-sectional images, fibers were cut with a sharp razor blade.

3.2.7 Enzyme activity

The activity of ALP was detected by photometric determination of the substrate conversion. The colorless substrate p-nitrophenylphosphate (pNPP) is converted by ALP to the yellow colored product p-nitrophenol (pNP), which has a absorbance peak at a wavelength of 405 nm and can be measured with UV-Vis Spectroscopy (Synergy Mx Multiplate Reader). Based on the measured absorbance, the pNP-concentration was calculated using a previously acquired pNP-calibration curve.

Immobilized Enzyme

For immobilized enzyme, a circular PEC film with a diameter of 15 mm or a hollow fiber with a length of 2 cm was cut and incubated at 37 °C in 500 µL of a 0.2 mM AMP buffer solution with a pH of 9.8 for 30 min. Subsequently, the PEC sample was washed again with buffer solution and placed in a reaction tube with 500 µL AMP buffer and 250 µL pNPP substrate solution. The reaction was incubated at 37 °C and 100 µL samples were taken at

determined time intervals. To prevent further reaction, each sample was mixed with 50 μL of 3 M NaOH solution. Measurements were carried out four times and averaged.

Free Enzyme

The enzymatic activity of free enzyme in solution was measured as follows. First, 500 μL of an 3 $\mu\text{g}/\text{mL}$ ALP solution in 0.2 mM AMP buffer was incubated for 30 min at 37 °C. Afterward, 250 μL of pNPP substrate solution was added, initiating the reaction. After dilution with the substrate, the reaction volume initially presents a total of 1.5 μg of ALP. This was determined to be the maximum amount of enzyme in the PEC film. Sample collection was performed analogously to the immobilized enzyme.

Thermal stability

The thermal stability was investigated at 37 °C, 50 °C, 60 °C, and 70 °C. PEC films and free enzyme were incubated for one hour at the given temperatures and then let to cool to room temperature. The enzymatic activity was then measured as described before.

Storage stability

For the investigation of storage stability, individual PEC films were prepared in advance and stored separately in 0.2 mL AMP buffer at 4 °C. For free enzyme measurements, reaction tubes containing 500 μL of a 3 $\mu\text{g mL}^{-1}$ ALP in 0.2 mM AMP buffer were prepared and stored at 4 °C. Enzymatic activity was measured as previously described.

Both thermal and storage stability are expressed as relative activity, which is defined as the actual activity divided by the activity measured under initial conditions (37 °C, week 0). The activity in itself is defined as the amount of product produced per unit time. In this case, it is calculated as follows:

$$U = \frac{\Delta C_P}{\Delta t} \cdot V_R \quad (3.1)$$

with U as the enzyme activity, Δc_P as the change in product concentration, Δt the time in which the concentration change was recorded, and V_R the reaction volume.

3.2.8 Membrane module fabrication

The membrane modules used for the different measurements consisted of one, two, or three hollow fibers inside polyethylene tubing (10 mm outer diameter) connected with two T-shaped IQS push-in fittings. The fibers were fixed in place with a layer of hot glue and a layer of a 2-component epoxy adhesive (UHU Plus Schnellfest). The effective length of the modules was 20 cm.

3.2.9 Pure water permeance

The pure water permeance (PWP) was determined by measuring the trans-membrane flux resulting from a constant TMP of 1 bar in dead-end mode. Water was permeated inside-out, and the permeate was collected and its weight recorded over time. The PWP was measured with three modules for each fiber batch. The standard deviation of the measurement is represented in the resulting error bars.

3.2.10 Burst pressure

The burst pressure of a single fiber was determined by slowly pumping water through the membrane and continuously increasing pressure until the sample burst. For this, single fiber membrane modules were constructed similarly to the modules used for filtration experiments. The pressure was applied from inside the fiber and manually increased with a hand pump. The fibers were measured in a wet state and at room temperature.

3.2.11 Molecular weight cut-off

The molecular weight cut-off (MWCO) is defined as the molecular weight of a solute that is retained by 90 % by the membrane. For the determination of MWCO, a solution containing 1 g/l each of PEGs with different MW (200, 400, 600, 1000, 1500, 4000, and 6000 g/mol) in DI water was prepared. Hollow fiber membrane modules were arranged in a cross-flow configuration and the feed solution was recirculated with a gear pump (MCP-Z, ISMATEC). The measurements were made at a TMP of 3 bar in an inside-out configuration. Permeate and retentate samples were collected after steady-state conditions were achieved. Size-exclusion chromatography by using an Agilent 1200 system to measure PEG concentration. The retention of each PEG was calculated with the permeate and feed concentrations ($C_{permeate}$ and C_{feed}):

$$R = 1 - \frac{C_{permeate}}{C_{feed}} \quad (3.2)$$

3.2.12 Enzyme immobilization stability in continuous use

Repeated rinsing and activity measurements were performed to investigate whether the ALP remains immobilized after continuous use. Hollow fiber modules with two fibers were used. First, the hollow fiber modules were rinsed in cross-flow mode using a gear pump (MCP-Z, ISMATEC) at 1 bar overnight. On the following day, activity measurements were conducted. Here, the substrate solution was filtered inside-out in dead-end mode with a volumetric flow rate of 8 μ L/min using a syringe pump (PHD/ULTRA of HARVARD APPARATUS). The permeate was directly collected in a reaction tube to prevent further contact with the hollow fiber. After 3 h, the collected permeate was analyzed as previously described. A rinsing cycle followed by the activity measurement encompasses one use cycle. In total, eight use cycles were conducted.

Enzyme activity is defined as the rate of the catalyzed reaction in terms of the amount of product produced in a certain time. In this case, it is ex-

pressed as nanomoles of product formed per minute. The activity was calculated using the following formula:

$$U = c_P \cdot \dot{V} \quad (3.3)$$

with U as the enzyme activity, c_P as the product concentration, and $\dot{V}$ as the volumetric flow rate of the substrate solution flowing across the wall.

3.3 Results and Discussion

First, the feasibility of ALP immobilization in PDADMAC:PSS PEC flat films is demonstrated. The enzymatic activity of immobilized ALP is compared against that of the free enzyme, and the effect of the PEC matrix on thermal and storage stability is evaluated. Then, the fabrication of self-supporting, mechanically stable hollow fiber membranes is described. Here, the improvement of the spinning process and the resulting morphology of the fibers are discussed. In addition, separation performance is investigated through cut-off and pure water permeance measurements, and repeated activity measurements are used to test the stability of the enzyme during filtration.

3.3.1 Enzymatically active polyelectrolyte complex films

PEC films with and without enzymes were fabricated using polymer solutions S.1 and S.2 listed in Table 3.1. Detailed preparation conditions are described in Section 3.2.2. The enzymatic activity and storage and thermal stability of the fabricated films are discussed below.

Enzymatic activity

The enzymatic activity of the immobilized and free ALP was analyzed by incubating the films and/or the free enzyme in a buffer solution containing the substrate pNPP. ALP catalyzes the conversion of pNPP to the product pNP. Therefore, the increase in product concentration over time directly relates to enzymatic activity. Each data point is an average of four different samples. The results of the activity measurements are shown in Figure 3.2. The series of measurements contains the activity measurement of immobilized ALP in a PEC film and three reference measurements. As reference free enzyme, free enzyme with the addition of a PEC film, and a PEC film are considered.

The immobilized ALP is shown to continuously increase the concentration of pNP, demonstrating the successful immobilization of the enzyme. In

contrast, no product formation occurs when only a PEC film is introduced into the substrate solution. For the immobilized ALP, a constant increase in the slope of the product formation curve is observed, implying a gradual increase in enzyme activity between each measurement period. This is attributed to diffusive effects, as the substrate first needs to be adsorbed and absorbed, with the product finally diffusing back into the solution. The delay in product release is observed primarily in the initial stages, as the film becomes bright yellow. This suggests that the amount of pNP converted during the experiments is higher than that measured from the solution. On the contrary, the free enzyme shows faster substrate conversion, reaching an overall higher concentration of pNP for each measured point. However, with the addition of a PEC film in the free enzyme solution, the concentration of the product decreases, reaching the same concentration as the immobilized enzyme. The negatively charged product readily adsorbs to the PEC with a positive surplus charge. The latter positive overall charge of this specific PEC composition has already been discussed previously [Kamp2021a].

Stability enhancement of the immobilized enzyme

The main advantage of enzyme immobilization is the reusability and possible enhancement of stability in harsher environments. The thermal stability of the immobilized enzyme was investigated by incubating samples of both the immobilized enzyme in PEC and the free enzyme in solution at different temperatures for 60 min. The results of these measurements are presented in Figure 3.3 a). After incubation at 50 °C, the enzymatic activity slightly increases for the immobilized enzyme compared to the free enzyme. The thermal stability of ALP has previously been demonstrated to improve with its immobilization in compact PECs [Tira2013]. This is possibly due to the stabilization of the enzyme structure and a better retention of metal ions in the enzyme's active site which are crucial for the catalytic function.

After incubation at 60 °C, the immobilized enzyme retained 60 % of its initial activity compared to 40 % for the free enzyme. After incubation at 70 °C, both immobilized and free enzymes show no activity, indicating their denaturation.

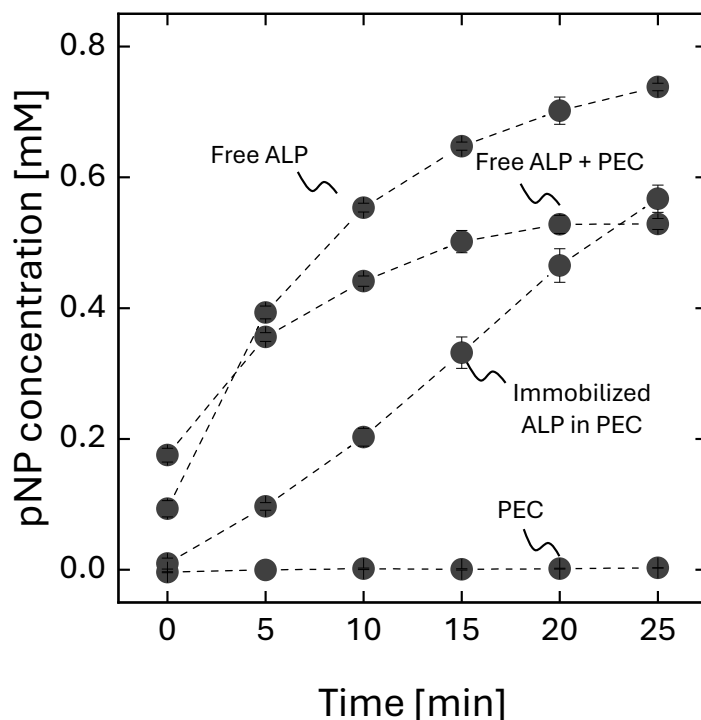


Figure 3.2: Enzymatic activity of immobilized ALP, free enzyme, free enzyme with a piece of PEC film, and a PEC film. Shown is the evolution of the product p-nitrophenol (pNP) from p-nitrophenol phosphate as a function of the time. Error bars indicate standard deviation.

In addition, the storage stability of the free and immobilized enzymes is investigated after 2 weeks of storage for a total of 8 weeks at 4 °C. The results of these measurements are presented in Figure 3.3 b. For immobilized enzymes, the enzymatic activity increases during the first 4 weeks of storage and then decreases to about 85 % of the initial activity. One possible explanation for the increase in enzymatic activity of the immobilized ALP is a change in the ionic strength of the enzyme environment due to the diffusion of remaining KBr ions from the PEC fabrication. Changes in ionic strength may lead to conformational changes in the ALP structure, leading to a temporary enhancement in activity. On the other hand, the free enzyme does not show an increase in enzymatic activity, and after 8 weeks, around 50 % of its initial activity is maintained.

Overall, these results demonstrate that immobilization of ALP in PDAD-MAC:PSS PECs provides a promising approach for enhancing the enzyme's

thermal and storage stability, thus improving their reusability with potential applications in harsher environments.

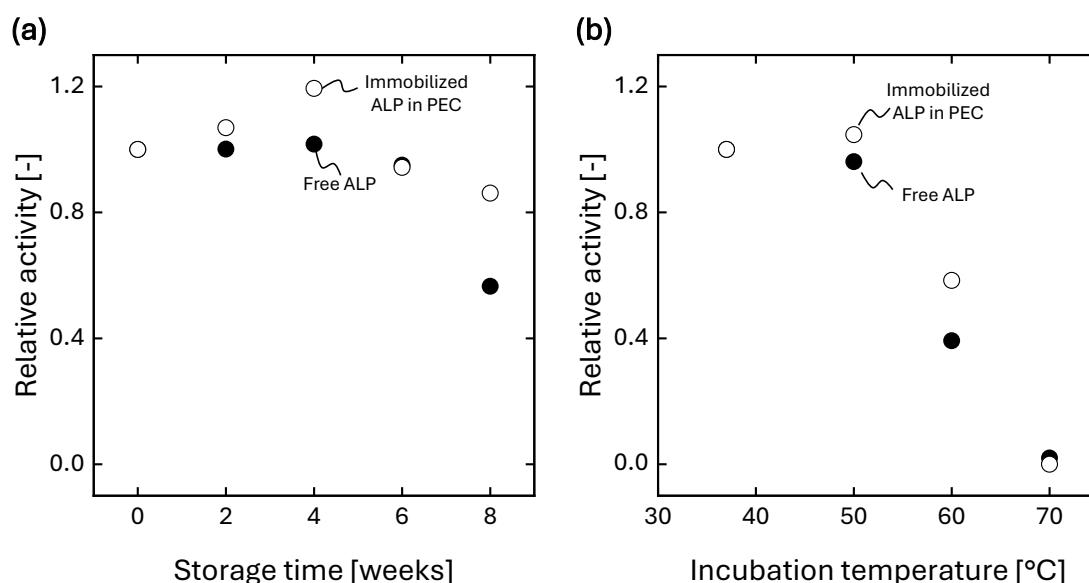


Figure 3.3: Relative activity of immobilized and free enzyme after incubation at various temperatures (a) and after several weeks in storage at 4 °C (b).

3.3.2 Fabrication of mechanically stable polyelectrolyte complex hollow fiber membranes

As demonstrated in the previous section, it is possible to immobilize enzymes in free-standing polyelectrolyte films. However, hollow fiber geometries provide higher packing densities and better hydrodynamic conditions than flat sheet membranes. Obtaining reproducible tubular or hollow fiber membranes remains a challenging research question, as already demonstrated by Emonds et al. and Baig et al. [Emon2021; Baig2022]. One main reason for this is the insufficient mechanical stability and the formation of defects in the selective layer, leading to non-reproducible results.

Optimization of membrane fabrication conditions

To address the formation of defects, adjustments were made to the conditions of salt-dilution-induced phase separation (SIPS). A suitable cross-

linker was chosen to enhance the mechanical stability of the membranes. Moreover, the composition and viscosity of the solution were modified, and the temperature adjusted. As a result, the membrane morphology could be finely tuned.

Increasing the viscosity of both the polymer and the bore solutions played a crucial role in preventing the formation of defects. This was achieved by raising the polymer concentration from 20 % (used for film fabrication) to 25 %, ensuring the extrusion of a continuous polymer jet that could withstand the tension of the pulling wheel.

Furthermore, the addition of glycerol to the bore solution increased its viscosity and also delayed the precipitation of PEC membranes. Previously, the addition of glycerol to the bore solution caused the formation of a thicker and denser selective layer, reducing the occurrence of defects [Emon2021]. However, despite the inclusion of glycerol in the bore solution, achieving consistent membrane performance proved challenging.

Here, the precipitation of the hollow fiber membranes is further delayed by reducing the temperature of the coagulation bath from room temperature (RT) to 0 °C. To visualize the effect of the coagulation bath temperature in the SIPS process, PEC films are cast from the polymer solution S.5. The films are immersed in coagulation baths at either room temperature (RT) or at 0 °C. The light transmittance across the film was recorded during the phase inversion. The results are shown in Figure 3.4, left. The sharper decrease in transmittance for the film precipitated at RT demonstrates faster precipitation than the film submerged in 0 °C.

Interestingly, no differences are observed in the morphology of the surface layer of the flat films presented in Figure 3.4, right. Contrary to expectations of a thicker surface layer resulting from delayed demixing, only slight variations in the pore size of the sub-structure are observed. Interestingly, the films casted at room temperature exhibited a more open structure. Additionally, both membranes showed a surface layer thickness of approximately 30 nm. However, when hollow fibers are fabricated in a coagulation bath at 0 °C, the permeance of pure water cannot be measured, indicating the formation of a defect-free membrane. This lack of measurable

permeance could be attributed to the low transmembrane pressure (TMP) used (0.1 bar). Higher pressures could not be used, as the fibers would burst at higher pressures. In contrast, hollow fiber membranes fabricated at RT showed high but inconsistent permeance even at low TMP (data not shown). Both a higher viscosity of the polymer solution and a slower salt diffusion rate (solvent exchange) can lead to slower precipitation [Stra1975; Hold2015] as seen in the light transmittance results. However, no significant effect on the membrane structure is observed in this case. Instead, an increase in viscosity prevents the deformation of the hollow fibers during fabrication, leading to the formation of defect-free membranes.

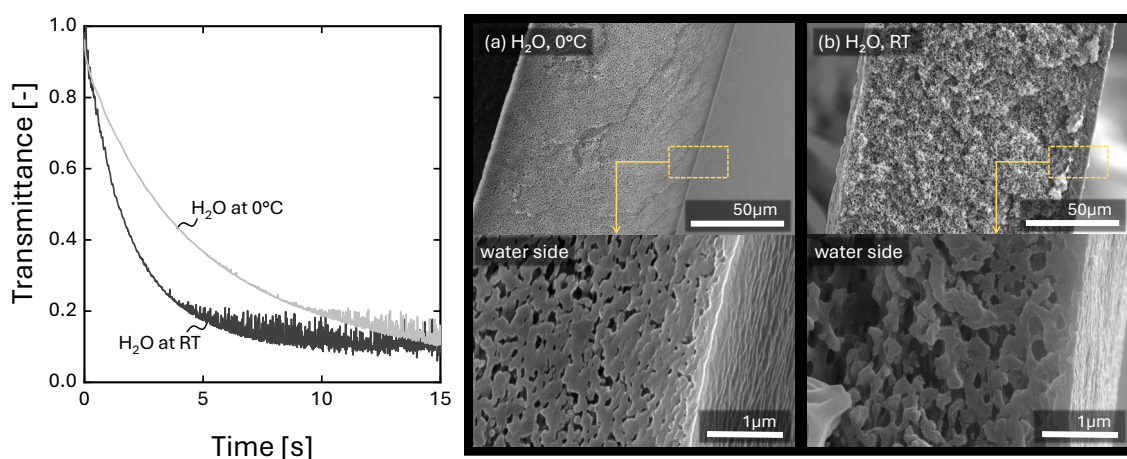


Figure 3.4: Coagulating rate defined by light transmittance measurements and cross-sectional images of PEC flat sheet films precipitated in H₂O at room temperature and 0 °C. Close-up image represents the side of the membrane in contact with water during coagulation.

However, an increase in mechanical stability was necessary. Zhang et al. described a method in which the backbone of polyelectrolyte chains is covalently linked using the DAS cross-linker and UV light regardless of charge or the presence of functional groups [Zhan2012]. Therefore, a subsequent photo-induced cross-linking of the fibers with DAS was performed.

Consequently, the hollow fiber membranes used in this study were spun in a coagulation bath at 0 °C using the polymer and bore solutions with glycerol introduced in Table 3.2. Additionally, if not stated otherwise, the hollow fiber membranes are cross-linked with DAS as described in Section 3.2.2. The characteristics of hollow fiber membranes with and without immobilized ALP

are discussed in the following sections.

Morphology

The intrinsic characteristics of manufactured hollow fiber membranes with and without immobilized enzymes are discussed. Table 3.3 lists the dimension and burst pressures of hollow fibers with ALP (HF.ALP) and without ALP (HF.ref), and with and without cross-linking with DAS and UV light. While all fibers have similar dimensions with inner diameters ranging from 375 to 480 μm , and wall thickness between 120 and 190 μm , no correlation is apparent between cross-linking or the addition of enzyme and fiber dimensions. The differences in dimension are instead due to the higher elasticity of PDADMAC:PSS complexes when there is a higher degree of salt ions acting as doping agents [Sham2012]. During and directly after fabrication, any involuntary applied force may lead to the elongation of the fibers. Indeed, previous experiments demonstrated that the fibers are fully set only after soaking in water for at least 20 min when most of the salt ions have been removed. As anticipated, cross-linking of the fibers significantly increases their mechanical stability, as demonstrated by an increase in burst pressure. As mentioned in the previous section, membranes without cross-linking burst below a pressure of 0.1 bar. However, after cross-linking, both HF.ref and HF.ALP burst pressures are above 6 bar.

Table 3.3: Dimensions and burst pressures measured for the fibers spun, $n=3$. The dimensions are determined by the dimension tool in the digital microscope.

	HF.ref -	HF.ref.c (cross-linked)	HF.ALP -	HF.ALP.c (cross-linked)
ID [μm]	467 ± 17	406 ± 3	375 ± 4	479 ± 7
Wall tickness [μm]	144 ± 5	194 ± 7	171 ± 2	117 ± 1
Burst pressure [bar]	< 0.1	6.2 ± 1.09	< 0.1	7.0 ± 0.84

FESEM images of PEC hollow fibers with and without immobilized ALP are depicted in Figure 3.5. All hollow fibers feature an asymmetric sponge-like porous structure with both a dense lumen layer (b.1-4) and shell layer

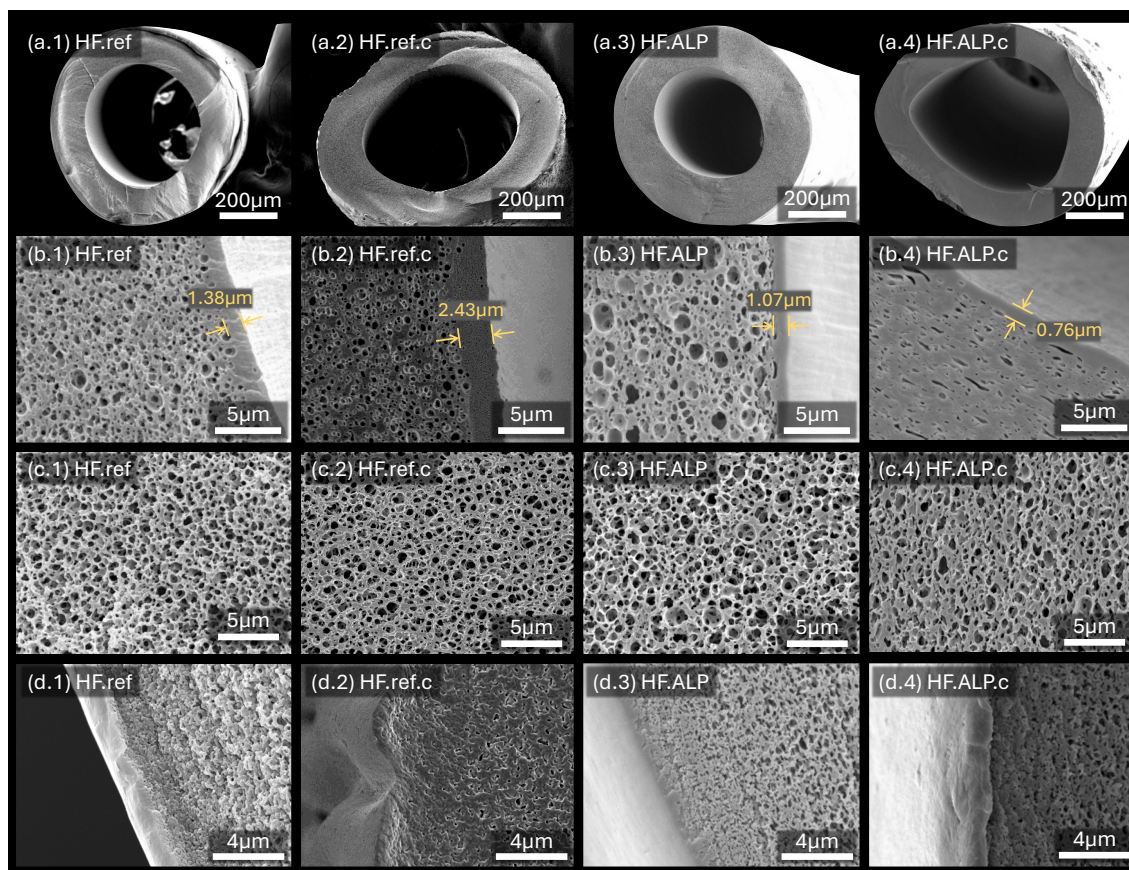


Figure 3.5: Cross-sectional FESEM images of PDADMAC:PSS hollow fibers. Reference fibers HF.ref and DAS cross-linked reference fibers HF.ref.c. Fibers with immobilized ALP HF.ALp and DAS cross-linked fibers with immobilized ALP HF.ALp.c; Cross section (a.1-4), lumen side (b.1-4), middle section (c.1-4), and shell side (d.1-4).

(d.1-4). However, the lumen layer is distinctly thicker, ranging from 0.8 to 2.4 μm . It has a porous substructure with larger, but similar-sized pores. This results from a delayed demixing of the lumen layer caused by the use of glycerol in the bore solution. A similar structure was already observed previously [Emon2021]. The shell layer, which coagulated in water, presents a structure similar to the flat sheet membranes discussed in Figure 3.4. A thin dense layer is observed that steadily evolves into larger interconnected pores.

Confocal microscopy was conducted to evaluate the enzyme's distribution in the hollow fiber. Reference fibers without enzymes are compared with fibers fabricated using fluorescence-labeled ALP. The fibers used were not cross-linked, as the NHS fluorescein easily bleaches after exposure to

light. The images obtained of the cross-section of the fibers are depicted in Figure 3.6. As expected, no fluorescence signal is observed for the fibers without ALP. In contrast, a uniform signal can be detected for fibers with ALP, demonstrating that the enzyme is homogeneously distributed across the polymer matrix.

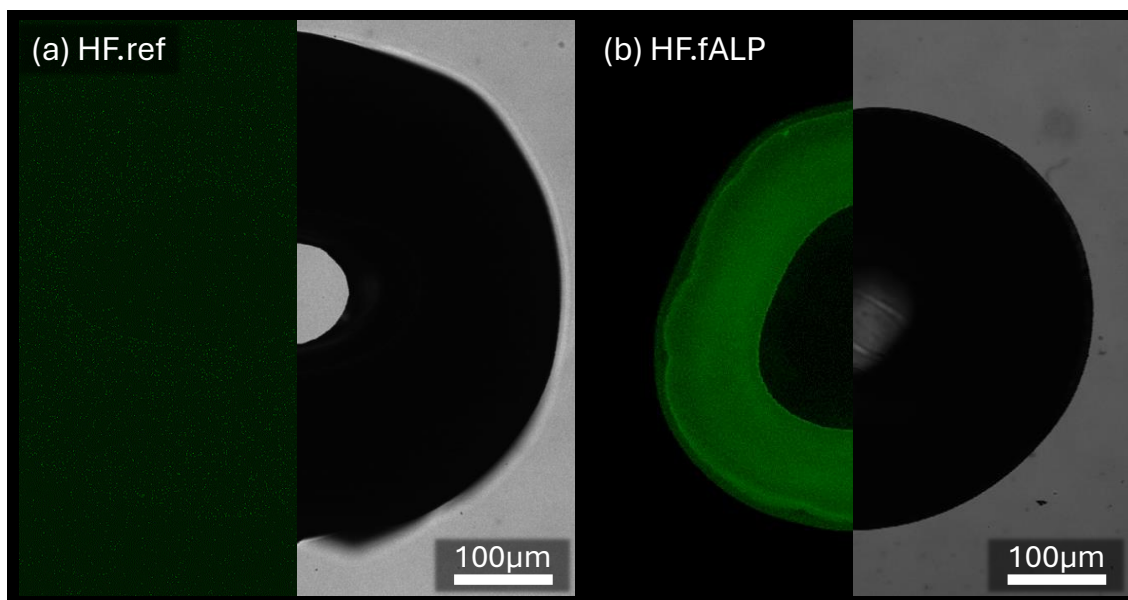


Figure 3.6: Fluorescence microscope images of the cross-section of non cross-linked hollow fiber membranes (a) HF.ref without incorporated enzyme fluorescent labeled ALP and (b) HF.fALP with fluorescent labeled ALP.

Molecular weight cut-off and pure water permeance

The results of the measurements of the molecular weight cut-off (MWCO) and pure water permeance (PWP) of the hollow fiber membranes with and without immobilized ALP are presented in Figure 3.7. We observe a difference in 1 LMH/bar water permeance in membranes fabricated with and without ALP. We argue that the lower water permeance of HF.ref.c is due to its greater wall thickness compared to HF.AL.P.c (see Table 3.3). In the case of solute rejection, the MWCO measured for both membranes are 520 and 420 Da for HF.ref.c and HF.AL.P.c, respectively. However, it cannot be concluded with certainty whether the differences in MWCO are due to the presence of the enzyme.

These findings regarding the membrane's performance have implications for potential applications. One promising use of immobilized ALP is in the removal of toxins from blood during hemodialysis treatment [Pete2014]. However, hemodialysis necessitates ultrafiltration type membranes with a MWCO of around 20 kDa [Hage2023], which is significantly larger than the cut-off of the PEC hollow fibers. Nonetheless, by selecting an appropriate enzyme, nanofiltration can also be considered. For example, prior studies have demonstrated the simultaneous degradation and removal of micro-pollutants in wastewater through the immobilization of oxidative enzymes on nanofiltration membranes [Li2020; Zhan2021].

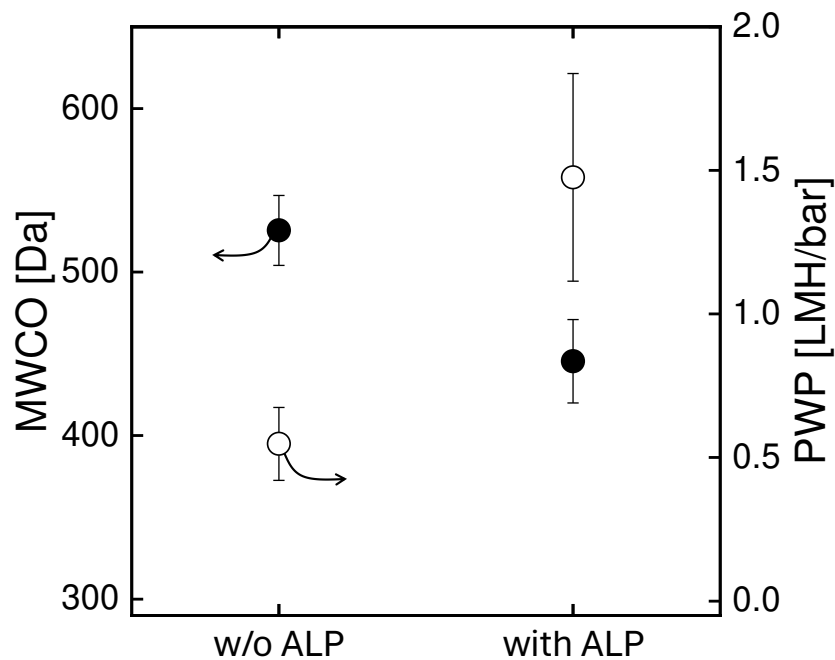


Figure 3.7: Molecular weight cut-off (MWCO) and pure water permeance (PWP) of the polyelectrolyte complex hollow fiber membrane with and without incorporated enzyme ALP. The MWCO was determined in cross-flow mode at a transmembrane pressure of 1 bar. The PWP measurement was conducted dead-end at a TMP of 1 bar.

Activity and enzyme stability of polyelectrolyte complex hollow fiber membranes

As described in Section 3.3.1, it is possible to obtain biocatalytic PEC flat sheet membranes with activities similar to those of the free enzyme. To translate these supported biocatalytic films to self-supporting biocatalytic hollow fiber membranes, the photo-induced cross-linking with DAS and UV light is crucial. However, enzymes are also susceptible to denaturation by UV irradiation [Mirs1936], and therefore the influence of the cross-linking process was investigated.

We measured the enzyme activity of hollow fiber membranes with immobilized ALP with and without cross-linking, with the results presented in Figure 3.8 (a). An increase in pNP concentration for fibers with and without cross-linking is observed. However, the product concentrations obtained with the irradiated membranes are about 30 % lower than the untreated fibers. As the intensity of the irradiation is highest on the outside of the fibers, it is theorized that most of the denatured enzyme is located on the shell of the fiber. Nonetheless, a substantial amount of active enzyme remains in the lumen and the midsection. The present results reflect the enzyme's susceptibility to UV light; therefore, shorter irradiation times and alternative cross-linkers should be considered in future work.

Subsequently, filtration experiments were conducted with cross-linked hollow fibers with immobilized ALP to test the stability of the enzyme over several reuse cycles. Here, the substrate was permeated dead-end through the fiber wall, and permeate samples were collected over time. The first measurement was conducted directly after cross-linking. Afterwards, the fibers were thoroughly rinsed with DI-water overnight, and the activity measurement was conducted again. In total, eight use cycles were performed. The results of these experiments are presented in Figure 3.8 (b). Two separate membrane modules were used with two fibers each. We observe an increase in activity after the first use cycle from around 0.8 nmol/min to 1.7 nmol/min. We attribute the lower activity in the first cycle to the improper wetting of the membrane, which then improved during the first washing cycle. A lower degree of wetting might lead to less substrate reaching the

enzyme, leading to lower product conversion. After the third use cycle, the activity stabilizes at around 1.3 nmol/min. This demonstrates that the ALP is effectively immobilized in the polymer matrix and does not leach during washing. Concluding, both mechanical stability and catalytic activity are combined in the PEC hollow fiber membranes.

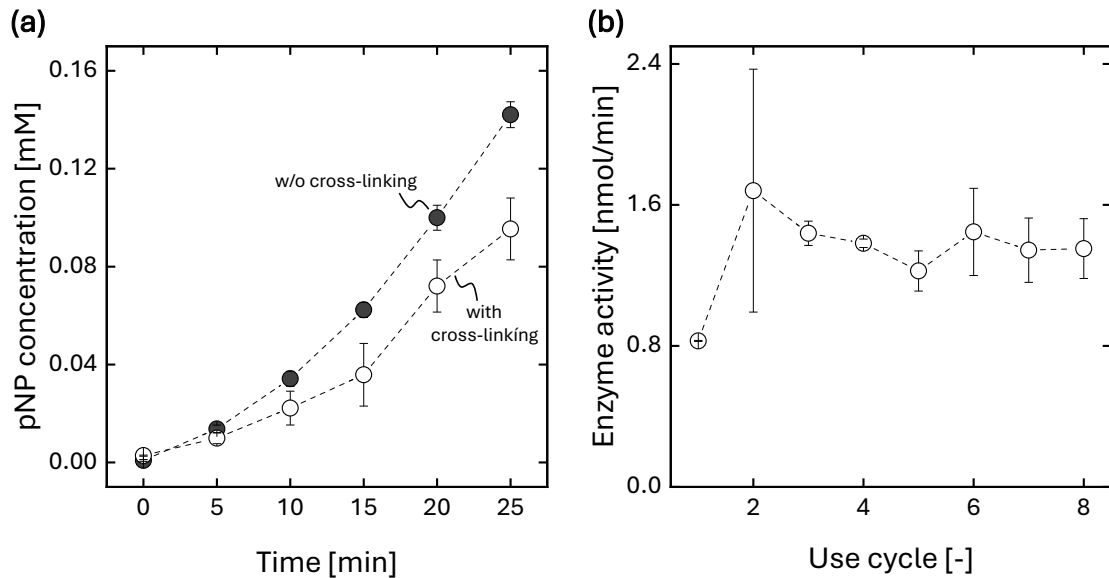


Figure 3.8: Enzymatic activity of PDADMAC/PSS PEC hollow fiber membranes containing ALP with and without cross-linking.

3.4 Conclusion

The reported polyelectrolyte complex hollow fiber membranes with an outer diameter of around 600 μm are free-standing, mechanically stable, and biocatalytically active. The membrane evolves via the salt-dilution-induced phase inversion (SIPS) process, which is an all-aqueous process. A polymer solution consisting of the polyelectrolytes PSS and PDADMAC, KBr, water, and the enzyme alkaline phosphatase (ALP) is precipitated in a coagulation bath at 0 °C. To achieve mechanical stability, we subsequently cross-linked the hollow fibers using UV light and the cross-linker DAS. Moreover, while cross-linking was achieved after fiber fabrication, the spinning process can be further optimized to include an in-line cross-linking. For example, by adding DAS directly to the polymer solution.

Experiments with flat films demonstrate that the PEC matrix enhances both the thermal and storage stability of the enzyme. Furthermore, we observed similar enzymatic activities for the free and immobilized enzyme, which demonstrates that the fabrication process does not hinder the enzyme activity.

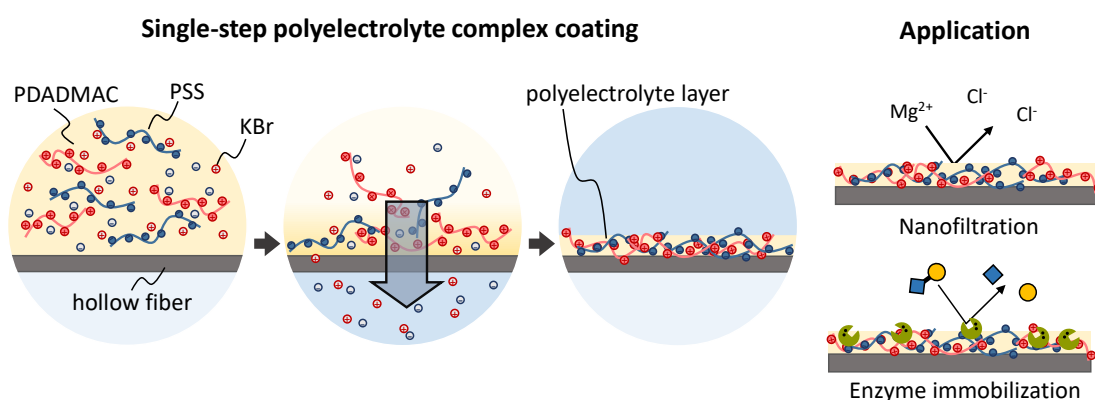
More significant is the fabrication of the free-standing hollow fiber membranes. Through improvements in the spinning process by the use of a coagulation bath at 0 °C and a 50 wt% glycerol/water bore fluid, we were able to achieve defect-free membranes with a MWCO between 400 and 600 Da and a PWP between 0.5 and 1.5 LMH/bar, demonstrating nanofiltration characteristics. Subsequent cross-linking significantly increases the mechanical stability of the fibers, which is demonstrated by an increase in the burst pressure from 0.1 to 6 bar. Furthermore, the enzyme retained 70 % of its initial activity after exposure to UV light during cross-linking. We hypothesize that only enzymes on the shell of the hollow fiber, which are directly exposed to UV light, are denatured. However, by measuring the activity of the fibers between the extensive washing steps, we demonstrated that the enzyme is stably immobilized inside the polymer matrix. The direct integration of enzymes in the polymer solution eliminates the need for a less effective and more time-consuming post-treatment. In fact, successful im-

mobilization of ALP opens interesting possibilities in the field of dialysis and the treatment of sepsis related acute kidney injury.

Overall, the development of defect-free mechanically stable PEC-based hollow fiber membranes is a major advancement with the potential for a wide range of applications. Incorporation of the enzyme ALP shows the versatility and robustness of the salt-dilution-induced phase separation (SIPS) process. By further tweaking the SIPS process and cross-linking, we assume that a broad range of hollow fiber membranes with diverse characteristics can be fabricated.

4

Modification of hollow fibers by a single-step polyelectrolyte complex coating



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4.1 Introduction

For the fabrication of tight ultrafiltration, nanofiltration and open reverse osmosis membranes, polyelectrolytes (PE) are widely used, also for surface modification, and functionalization in general [Durm2021]. For this, the Layer-by-Layer (LBL) method is predominantly used. Introduced by Decher et al. in 1997, the LBL technique uses the principle of polyelectrolyte complexation to modify a substrate surface through the step-by-step deposition of oppositely charged polyelectrolyte layers [Dech1997]. This has allowed to tailor the properties of a membrane to obtain, for example, nanofiltration (NF) membranes that achieve competitive fluxes and rejections to polyamide commercial membranes [Hong2007; Kamp2021b]. However, multiple coating and washing steps make LBL coating an intensive, time-consuming process, and efforts have been made to streamline PE incorporation directly during the membrane fabrication process [Emon2022; Moha2021].

Additionally, further functionalization can be achieved through the immobilization of enzymes in the PE layers. This can be achieved by either the entrapment of the enzymes between the PE layers, by enzyme adsorption via electrostatic interactions, or by the covalent binding of the enzyme using cross-linkers [Dizg2018; Li2018; Li2020; Yong2015; Smul2011; Sarm2017].

In recent years, more understanding has been gained in the phase behavior and the interaction between oppositely charged PEs in aqueous environments. Indeed, Chapter 3 demonstrated the fabrication of polyelectrolyte complex (PEC) hollow membranes with biocatalytic properties. This was achieved by dissolving the strong polyelectrolytes polydiallyldimethylammonium chloride (PDADMAC) and polystyrene sulfonate (PSS) with sufficiently high potassium bromide (KBr) and water. The precipitation of this solution by the controlled removal of the KBr ions in a water coagulation bath. Since this process is fully aqueous, it enabled the direct immobilization of enzymes which would otherwise degrade in the presence of organic solvents used in traditional membrane fabrication.

So far, membrane fabrication via aqueous phase separation has only been used for the formation of membranes consisting of a full PEC matrix [Kamp2021a; Emon2021; Durm2020; Sadm2019; Baig2020; Baig2021; Baig2022]. However, in their hydrated state, PEC membranes lack rigidity, which means they can be deformed under pressure, leading to defect formation, compaction, and low permeability if no support structure is used [Emon2021]. Recently, Li et al. developed a single-step PEC coating method that utilizes a polymer solution containing polyethylenimine (PEI), PSS and NH_3 . The evaporation of NH_3 causes the pH of the solution to drop, leading to the protonation of PEI and, thus, the complexation of PEI/PSS. However, this method has only been applied to flat substrates. [Li2021; Li2022]

This work aims to overcome the main drawbacks of both LBL and free-standing PEC membranes by directly coating a hollow fiber support with a homogeneous PEC solution. By analyzing the phase behavior of PECs under various ionic strengths and PE concentrations, three simple coating mechanisms are proposed, each differing in how phase separation is approached. The effectiveness of these mechanisms is evaluated in relation to the formation of a selective NF layer. Additionally, the potential for further membrane functionalization is explored by incorporating enzymes into the coating solution, enabling direct entrapment during the coating process.

4.2 Materials and Methods

4.2.1 Materials

PSS with high molecular weight (MW) (average MW $\sim 1,000,000$ powder) and low MW (average MW $\sim 200,000$; 30 wt % in H_2O), and PDADMAC with high MW (average MW $\sim 400,000$ -500,000; 20 wt % in H_2O) and low MW (average MW $\sim 200,000$; 20 wt % in H_2O) are supplied from Sigma Aldrich. Potassium bromide (KBr) (99 %) was purchased from Alfa Aesar. Alkaline phosphatase (ALP) from bovine intestinal mucosa (lyophilized powder, ≥ 10 U/mg), p-nitrophenol (pNP) (10mM solution), 2-Amino-2-methyl-1-propanol (AMP) (≥ 95 %), Tris-Buffered saline solution (TBS), sodium hydroxide (NaOH) (≥ 98 %) are supplied by Sigma Aldrich. For the enzyme activity measurements, a ready to use p-nitrophenylphosphate solution (pNPP) was purchased from Sigma Aldrich. For ALP labeling, NHS-fluorescein (5/6-carboxyfluorescein succinimidyl ester) was purchased from Thermo Fisher and dimethylsulfoxid (DMSO) (≥ 99.5 %) was obtained from Carl Roth.

For the fabrication of nanofiltration membranes, a polyethersulfone (PES) hollow fiber support with an inner diameter of 0.8 mm and an average molecular weight cut-off of 10kDa was kindly provided by Pentair. For enzyme immobilization, a hollow fiber support with a nominal pore size of 20 nm and an inner diameter of 0.83 mm was provided by Pentair as well. Single fibers were used to build membrane modules with an active fiber length of 20 cm.

4.2.2 Membrane modification

Interfacial Complexation

For interfacial complexation, a film of PE-solution was deposited on the lumen's surface. This was done by filling the lumen with the solution, the removal of the bulk liquid using compressed air, and the subsequent rinsing with deionized (DI) water. A newly prepared membrane module, previously stored in 20 wt % ethanol-solution overnight and was subsequently rinsed

with DI water. To prevent uncontrolled complexation of PE, residual liquid in the lumen was displaced with a 2 M KBr-solution. The composition of the solutions used for the coating are given in Table 4.1.

After the preparation steps, a polyelectrolyte (PE) solution was pumped into the upright mounted membrane module with a syringe pump at a flow rate of 1 ml/min. After filling the module with PE-solution a valve at the top of the module was closed and the syringe pump activated again. The TMP build up during this dead-end step was monitored using a pressure gauge. After reaching 2 bar TMP, the pump was stopped, the valve was cautiously opened, and the PE-solution withdrawn by the syringe pump. To ensure no PE residues in the membrane's lumen except for a thin film on the surface, the module was detached and compressed air was blown through the module for 1 min. The flow rate of the air was adjusted to about 0.5 L min^{-1} . After mounting the module upright again, the PE film on the membrane's surface was complexated with DI water. In this step, DI water was filtered dead-end for at least 15 min with a constant pressure of 3 bar. Coated membranes were stored in DI water at room temperature until further use.

Table 4.1: Composition of the coating solutions used for the interfacial complexation.

c_{PE} [wt.%]	c_{KBr} [mol _{KBr} /l _{H₂O}]	PSS:PDADMAC ratio [monomer ratio]	PE size -
2.5	2.0	1	high MW
10	2.0	1	low MW

Diffusive desalination

Diffusive desalination was based on continuous circulation of water through shell of the hollow fiber while the membrane's lumen is filled with an over-critical PE-solution. KBr was expected to diffuse through the membrane resulting in the complexation of PE on the membrane's surface. The membrane module was prepared by storing it in ethanol, followed by rinsing with DI water and KBr solution, as described in the previous method. The mem-

branes were coated according to the specified preparation steps. A distinction is made between membranes used for nanofiltration (NF) experiments and those used for enzyme immobilization. The composition of the coating solutions is provided in Table 4.2.

Table 4.2: Composition of the coating solutions used for the diffusive desalination. Solutions without ALP were used for the coating of nanofiltration membranes. Solutions containing ALP were used for enzyme immobilization.

C_{PE} [wt.%]	C_{KBr} [mol _{KBr} /l _{H₂O}]	PSS:PDADMAC ratio [monomer ratio]	PE size -	C_{ALP} [mg/ml]
0.1	2.0	1	high MW	-
0	2.0	1	low MW	5
0.1	2.0	1	low MW	5
0.2	2.0	1	low MW	5
1.0	2.0	1	low MW	5

For membranes prepared for nanofiltration experiments, the lumen of the hollow fiber was filled with the polymer solution. Then, DI water was flushed across the shell side of the fiber with a volumetric flow rate of 120 or 1200 g h⁻¹ for 40 min. Afterwards, the excess polymer solution was removed by pressurized air and DI water was filtered dead-end for at least 15 min with a constant pressure of 3 bar. Coated membranes were stored in DI water at room temperature until further use.

For membranes used for enzyme immobilization, the PE solution was recirculated to facilitate more enzyme adsorption. Following the preparation steps, the membrane's lumen was attached to a peristaltic pump, which was continuously drawing PE-solution from a reservoir of 10 mL and pumping it through the lumen at a flow rate of 10 mL min⁻¹. After flowing through the lumen, the solution was led back into the reservoir. As soon as the PE-solution was in continuous circulation, a second peristaltic pump was started for a DI water counterflow through the shell at 20 mL min⁻¹. The coating was stopped after 30 min by removing the PE-solution intake from the reservoir and subsequently pumping the PE-solution out of the membrane's lumen. Immediately afterwards, a syringe pump with DI water was

attached to the lumen and remaining residues of coating solution were complexated by pumping 50 mL DI water at a flow rate of 20 mL min^{-1} through the lumen. For storage Tris-buffered saline (TBS) was rinsed through the lumen and filled in the shell. When not in use, the modules were stored at 5°C .

Polyelectrolyte concentration

This method a polymer solution with concentration below the overcritical salt concentration was prepared. After the solution precipitated into a polymer rich and a polymer lean phase, the polymer lean phase was carefully collected and used as coating solution.

The composition of the solutions used is given in Table 4.3. Two different coating fluxes were used (75 and 100 LMH) and three different end pressure were set (6, 12, and 20 bar).

Table 4.3: Composition of the coating solutions used for the polyelectrolyte concentration. The suffix *i* refers to the concentration of the initial polymer solution. The suffix *lean* refers to the resulting polymer lean phase after phase separation.

$C_{PE,i}$ [wt.%]	$C_{KBr,i}$ [mol _{KBr} /l _{H₂O}]	PSS:PDADMAC _i [monomer ratio]	size PE -	$C_{PE,lean}$ [wt.%]	$C_{KBr,lean}$ [wt.%]	PSS:PDADMAC _{lean} [monomer ratio]
10	1.5	1	high MW	0.8	15.7	0.11
10	1.85	1	high MW	1.3	17.6	0.5

Before coating, the membranes were prepared as described previously. For the coating, the polymer solution was filtered in dead-end with a determined flux until a desired TMP was reached. Then, the excess solution was removed by pressurized air followed by filtration with DI water in dead-end for at least 15 min at 3 bar.

4.2.3 Thermogravimetric Analysis

To determine the polymer and salt content of the polymer-lean phase used for the polyelectrolyte concentration coating, thermal gravimetric analysis (TGA) was performed using a Simultaneous Thermal Analyzer STA6000

(Perkin Elmer Inc., USA). The mass of the dried sample is measured over time as the temperature changes. To allow comparison, all compounds were measured separately before. The samples were heated from room temperature to 600 °C with a heating rate of 10 °/min.

4.2.4 NMR spectroscopy

Proton NMR spectroscopy (Bruker Advance 300 MHz spectrometer) was used to determine the ratio of the polyelectrolytes in the polymer-lean phase. Therefore, the relative integrals of the individual PE compounds were compared. Samples containing ca. 30 mg PEC were dried at 100 °C to remove water completely. The dried samples were then dissolved in D₂O. Additional KBr was added depending on the relative KBr content in the dried sample.

4.2.5 Enzyme fluorescein labeling

Fluorescein-labelled ALP was prepared by solving 50 mg mL⁻¹ of ALP in 1 mL PBS and 10 mg/l NHS-fluorescein in 266 µl DMSO. Subsequently, both solutions were mixed in a reaction tube. The tube was incubated for at least 2 h in a fridge at 4 °C. Afterwards, the solution was centrifuged in an Eppendorf centrifuge 5415 R at 13000 rpm for 2 h with Ultracell PL-30 tubes. The samples were rinsed three times with 600 µl of PBS and subsequent centrifuging for 30 min. Thereafter the supernatant was removed, and the fluorescein-labelled ALP (f-ALP) found on the filter was redispersed two times with 750 µl PBS. The redispersed enzyme was collected, resulting in a total of 1.5 mL with 30 mg/mL f-ALP.

4.2.6 Membrane characterization

Salt rejection

Rejection experiments were performed in a cross-flow set-up at a trans-membrane pressure (TMP) of 2 bars. A 5 mmol L⁻¹) MgCl₂ dissolved in DI-water was used. Salt rejection was calculated with:

$$R = 1 - \frac{\sigma_P}{\sigma_F} \quad (4.1)$$

where σ_P and σ_F denote the measured conductivity of the permeate and the feed respectively.

Permeability

Pure water permeability (PWP) was determined by measuring the trans-membrane flux resulting from a constant TMP in dead-end mode. The experiment was prepared by connecting a hollow fiber module to a water tank pressured by compressed air. The TMP was controlled using a valve and a pressure gauge in front of the module. The permeability P was calculated as follows:

$$P = \frac{V}{S_A \cdot \Delta t \cdot \Delta p} \quad (4.2)$$

with V as the collected volume over a specified time period, S_A the membrane's surface area, Δt the measured time, and Δp the applied TMP.

Enzyme activity

The used assay for determining enzyme activity was based on the de-phosphorylation of para-nitrophenylphosphate (pNPP) to para-nitrophenol (pNP), which is catalyzed by ALP. 15 mL of pNPP-solution were used as substrate. With a peristaltic pump, the solution was continuously recirculated from a light protected reservoir through the membrane's lumen at a flow rate of 20 mL min^{-1} . Sample pairs were taken from the reservoir right before the experiment and in intervals ranging from 5 min to 30 min over up to 1.5 h. Each sample pair comprised a) 200 μL that were pipetted in an Eppendorf tube with 100 μL of 3 M NaOH (stop solution) and b) 200 μL that were pipetted in an Eppendorf tube without NaOH. After 15 min min NaOH was added to sample b as well. After the experiment 50 μL of each

Eppendorf tube were pipetted on a well plate, diluted with 150 μL DI water, and absorbance was measured at a wavelength of 405 nm using a BioTek Instruments Synergy Mx. Based on the measured absorbance the pNP-concentration was calculated using a previously acquired pNP-calibration curve. By calculating the increase in concentration between sample a and b of the same pair, the activity caused by leaked enzyme was determined. The increase in concentration between samples a of two different pairs was used to calculate the total enzyme activity. By subtracting leached enzyme activity from total activity, the immobilized enzyme activity was calculated. The loss of volume and product due to sampling was taken into account. The obtained enzyme activity was related to the active membrane area.

Microscopy imaging

Field emission scanning electron microscopy (FESEM) was used to acquire detailed pictures of the membrane surface. The samples were prepared by deposition in a 50 wt % solution of glycerol in water overnight. Subsequently, the hollow fibers were broken in liquid nitrogen for recordings of the cross-section or cut in half longitudinal with a razor blade for recordings of the surface. To completely remove water residues, the samples were freeze-dried for a week and stored in a desiccator. The final recording was done externally Hitachi S4800.

Modules were prepared for confocal microscopy by coating with one of the methods explained above and substituting 0.5 mg mL^{-1} of the ALP by f-ALP. After coating, the membrane was removed, and confocal microscopy images were taken using a Leica SP8 FALCON.

4.3 Results and Discussion

4.3.1 Coating methods

This work examines three different methods that make use of the phase behavior of the PSS:PDADMAC:KBr system to achieve surface modifications of hollow fiber membranes. Since various studies have demonstrated the formation of PECs from a homogeneous PSS:PDADMAC solution using KBr, the same salt is employed in this study [Emon2022; Wang2014; Kamp2021a; Wang2015].

A schematic representation of the three methods denoted as interfacial complexation (IC), diffusive desalination (DDS), and polyelectrolyte concentration (PC) is shown in Figure 4.1. A simplified two-phase diagram of the PE:KBr system is included to represent the different stages of the coating process. The following section provides an overview of the three coating methods.

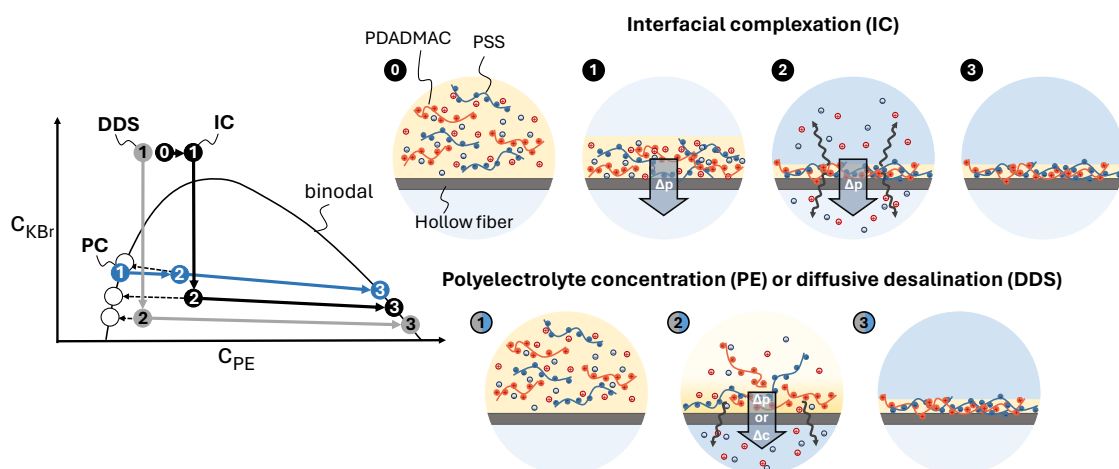


Figure 4.1: Schematic illustration of the coating methods interfacial complexation (IC), polyelectrolyte concentration (PC), and diffusive desalination (DDS). The salt and polyelectrolyte concentrations during the coating processes are depicted qualitatively in a simplified two-phase diagram (left) and correlated to a schematic visualization of the built-up of the polyelectrolyte complex coating (right)

Interfacial complexation - IC

In the interfacial complexation method IC, the membrane is first wetted with an overcritical PE solution. By filtering the solution through the membrane in a dead-end mode (0-1), the local concentration of PE increases directly at the membrane surface due to the size exclusion of PEs. Once the excess PE of the feed side is removed, water is filtered in dead-end mode, which rapidly decreases the concentration of KBr (1-2). Consequently, phase separation occurs, and a PEC layer forms (2-3).

Diffusive desalination - DDS

The diffusive desalination method DDS works similar to a dialysis process. An overcritical PE solution flows through the lumen of the fiber, while water flows on the shell side of the fiber. The difference in concentration between both solutions causes the PE and the salt to be drawn towards the shell side and water to be drawn towards the lumen. While salt molecules can pass through the membrane, PEs accumulate directly at the membrane surface. This process leads to a decrease in salt concentration (1-2), thus, precipitation of PEs occurs (2-3). This process is expected to be more gradual, as internal concentration polarization within the hollow fiber support acts as a salt storage during the desalination process. PEs accumulate at the surface until all PEs have formed complexes, either as a thin film or as PEC particles in the solution.

Polyelectrolyte concentration - PC

This method uses the supernatant from a PE solution prepared below an overcritical KBr concentration as a coating solution. Two solutions consisting of 10 wt % total polymer, 1:1 molar ratio of PSS:PDADMAC, and either 1.5 or 1.85 M KBr were used. In this case, the ratio and concentration of PEs in the supernatant can be tailored by the amount of KBr and PE used to prepare the initial polymer solution. This solution finds itself directly at the binodal of the two-phase diagram. Hence, by filtering the solution in dead-end mode at a defined transmembrane flux (75 or 100 LMH) until a certain

pressure is achieved (6, 12, or 20 bar), the PE concentration is increased directly at the surface of the membrane (1-2). This leads the solution to enter the solubility gap and precipitate into a thin PE layer (2-3).

4.3.2 Nanofiltration performance of PEC coated hollow fiber membranes

The apparent presence of the formed selective PE layer was evaluated by measuring the rejection of the positive divalent salt MgCl_2 and the change in water permeability of the coated membranes compared to an uncoated membrane. As both IC and PC require dead-end filtration of the PE solution during the coating, a tight ultrafiltration support from Pentair with a molecular weight cut-off of 10 kDa was used to ensure the complete retention of the PEs by the membrane. Moreover, the build-up of a positively charged PE layer is expected, as this PE system is particularly known for forming an excess positive charge [Kamp2021a]. Wang et al. argue that this is due to KBr being better at dissolving PDADMAC(Br) than dissolving PSS(K). Hence, PDADMAC is in a greater coiled state than PSS, resulting in a non-stoichiometric PE complexation.

Results of the salt rejection and permeability measurements are shown in Figure 4.3. The pentagon shaped marker denotes a PEC flat sheet membrane from [Kamp2021a]. The star marker denotes a LBL hollow fiber from [Kamp2021b]. Blue markers denote membranes coated with the method polyelectrolyte concentration (PC) with the polyelectrolyte-lean phase of a polyelectrolyte solution after phase separation. The polymer solution, which originates from polymer solutions with different KBr concentrations (1.85 and 1.5 M KBr), was filtered in dead-end mode until reaching a determined pressure (6, 12, 20 bar) at a fixed flux (75 and 100 LMH). Black markers denote membranes coated with the method interfacial complexation (IC). This method uses a thin layer of overcritical polyelectrolyte solution with varying molecular weight (low, high) and polyelectrolyte concentrations (2.5wt.%, 10wt.%) precipitated using water. Grey markers denote membranes coated using the diffusive desalination (DDS) method. In this

method, the diffusive removal of KBr from an overcritical polymer solution was achieved at two different water flow rates (120 and 1200 g/h).

For membranes coated with IC, a decrease in permeability is observed, attributed to the deposition of PE on the membrane surface. However, only membranes coated with the lower PE concentration exhibit significantly higher MgCl_2 rejection compared to an uncoated membrane.

Membranes coated with DDS showed no increase in salt rejection. However, decreasing permeability was observed with decreasing coating velocity. This is attributed to the deposition of large PE aggregates on the membrane surface that increase in size when the flowrate during coating increases (see Figure 4.2). During coating, salt ions diffuse out of the polymer solution and across the HF support through diffusion. At the shell side, salt is then removed through convective transport. However, if a lower water flow is used, a larger boundary layer forms and concentration polarization is increased. With a larger concentration polarization, the effective concentration difference across the porous support decreases, leading to a decrease in salt diffusion. This allows for a more extended coating process and the adsorption of smaller PEC precipitates in the surface. At larger flushing coating velocities, the desalination occurs more quickly, which may lead to the formation of larger aggregates in the bulk phase of the PE solution. These PEC aggregates possibly also adsorb to the HF surface. However, they are more easily washed away during measurement.

The most effective method is the polyelectrolyte concentration method PC. With this method, increasing salt rejections are achieved through the adjustment of the KBr concentration in the initial PE solution. Note that the KBr concentration simultaneously affects both PE concentration and ratio (see Table 4.3). Both vary in the resulting supernatants used for the coating. The higher KBr concentration leads to a supernatant with an overall higher PE concentration and a greater PSS:PDADMAC ratio compared to the lower KBr concentration. Furthermore, additional improvements in nanofiltration performance are observed by increasing the end pressure reached during coating and by reducing the coating flux.

It is theorized that with increasing end-pressure, larger amounts of PE

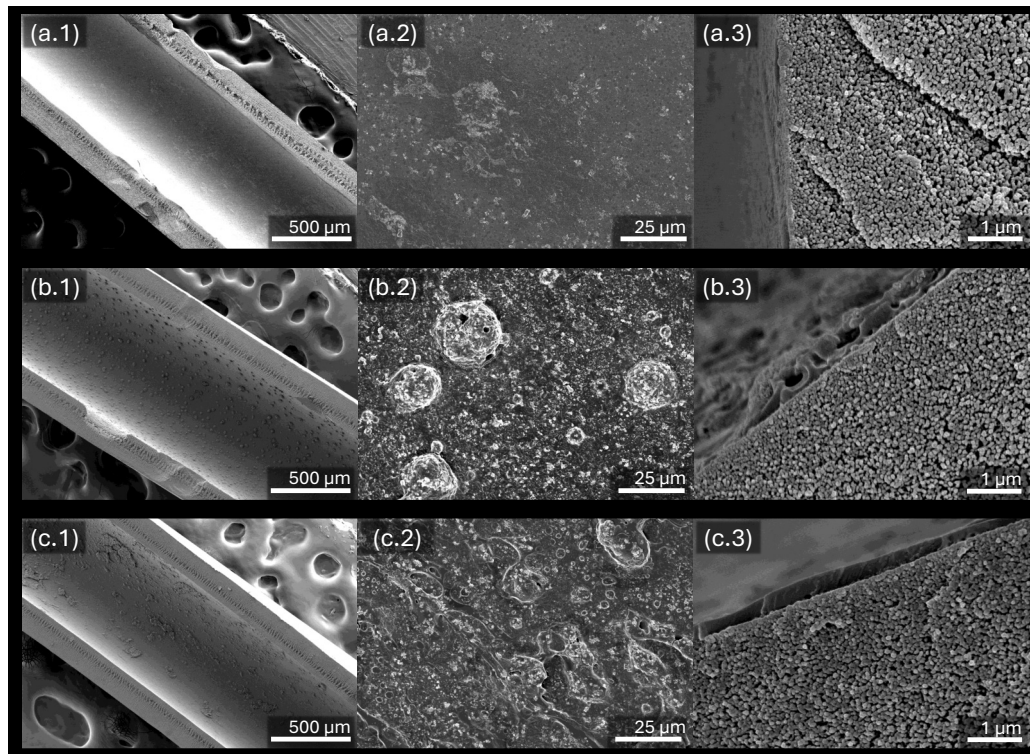


Figure 4.2: FESEM images of uncoated membrane (a.x) and membranes coated with diffusive desalination with 120 g h^{-1} water flow in the lumen (b.x) and 1200 g h^{-1} water flow in the lumen (c.x). x.1, 2: Lumen surface and lumen surface close-up, respectively. x.3: Lumen cross-section.

are deposited on the surface, which correlates with the decreasing permeability observed. Furthermore, a higher rejection was obtained if a coating flux of 75 LMH instead of 100 LMH was used. It is theorized that the PE solution does not precipitate instantly; instead, a meta-stable phase is first reached. This phase's stability depends on the difference between the local concentration and the equilibrium concentration, also known as the degree of supersaturation. In Figure 4.1, this can be denoted as the difference between points (2) and (1) for the PC coating method. If the difference is larger, the phase will precipitate faster [Mart2021]. It is speculated that by reducing the coating flux, the degree of supersaturation at any given location above the membrane surface is also reduced. This might eventually lead to a larger amount of precipitated PE as not only is the PE allowed to accumulate for longer, but the layer height in which it accumulates might be higher. However, further research needs to be conducted to confirm this theory.

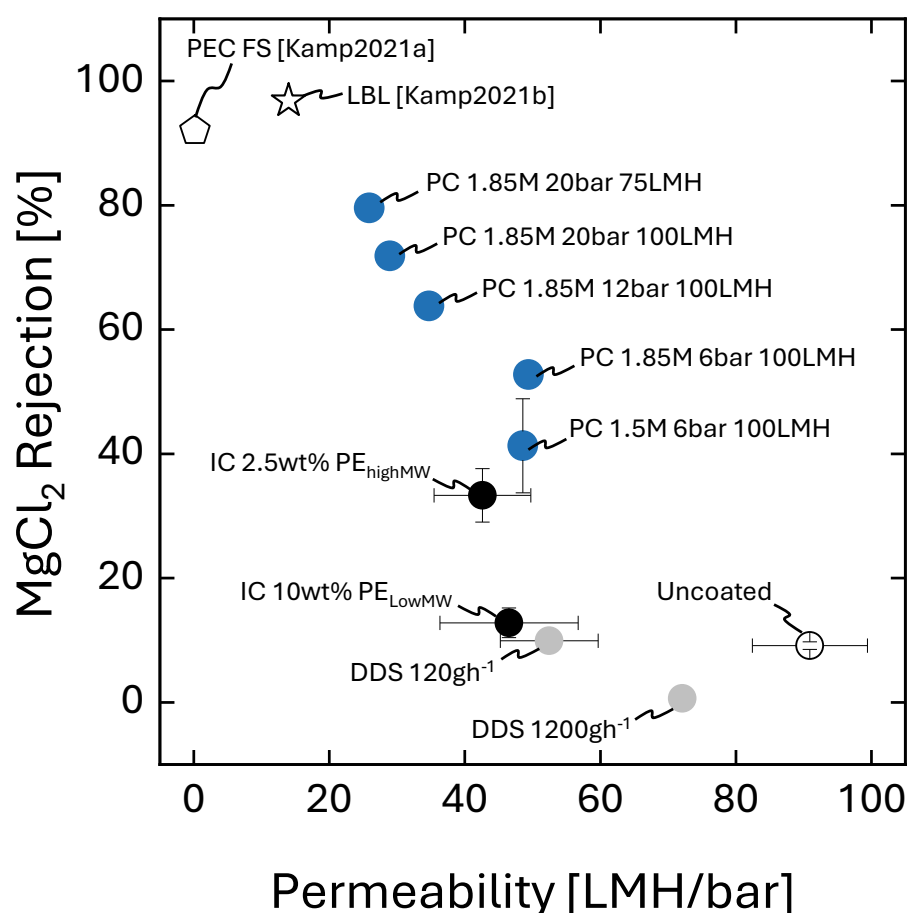


Figure 4.3: MgCl₂ rejection and permeability of polyelectrolyte complex coated hollow fiber membranes coated via IC, DDS and PC methods. Salt rejection was measured in cross-flow at 2 bar TMP with a 5 mmol/L MgCl₂ solution. Water permeability was measured in dead-end mode at 2 bar TMP.

4.3.3 Enzyme immobilization

A second relevant functional membrane may be achieved through the above-described membrane fabrication methodology. A single-step modification with PE is envisioned as a tool for immobilizing enzymes to fabricate biocatalytic membrane reactors.

The DDS method is proposed as an enzyme immobilization technique due to its simplicity and its ability to deposit a polyelectrolyte layer that can entrap enzymes. Furthermore, the versatility of the DDS method is evaluated by utilizing a different support membrane from the one used in Section 4.3.2. Despite the support membrane for enzyme immobilization having

larger pores, the PEs are not expected to infiltrate the porous structure, as no pressure is used to deposit the PE layer. Some alterations were introduced to the coating method to maximize enzyme use (see Section 4.2.2).

Figure 4.4 depicts the differences in morphology of the fibers coated without (d) PE and with 0.2 wt % (e). FESEM images of the lumen of the HF (a.1,e.1) demonstrate the deposition of sphere-like agglomerates of PE, indicating the presence of deposited material. Moreover, confocal imaging (d.2 and e.2) indicates the presence of enzyme on the lumen of the HF regardless if PE was used in the coating. This is expected, as enzymes, and proteins in general, readily adsorb to any surface. However, it is not possible to determine if the amount of immobilized enzyme is greater when PEs are used in the coating. For this reason, activity measurements were conducted.

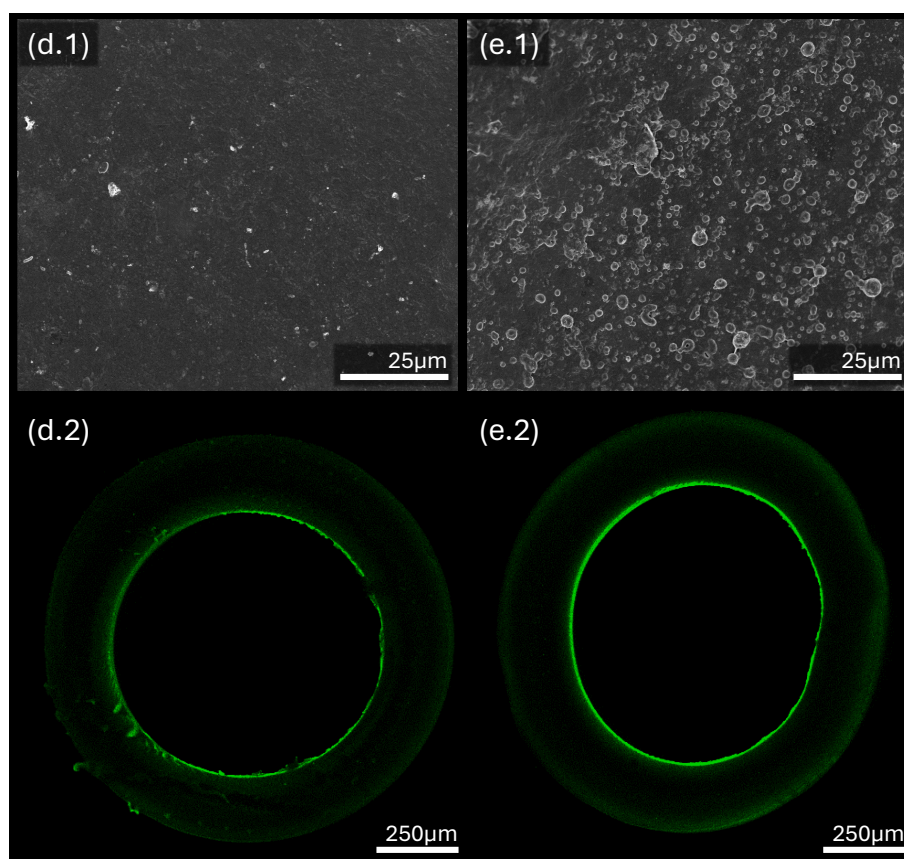


Figure 4.4: FESEM images of the lumen of hollow fiber membranes coated with 5 mg/ml ALP and 0 wt % (a.1 and a.2) or 0.2 wt % PE (b.1 and b.2). Confocal images of the cross section of hollow fiber membranes coated with 0.5 mg/ml fluorescent labeled ALP and 0 wt % (a.1 and a.2) or 0.2 wt % PE (b.1 and b.2).

Enzymatic activity was measured for the enzyme immobilized in the PE layer (immobilized ALP, filled circles) and for the enzyme that desorbed from the layer during the measurement (desorbed ALP, blank circles). The results of these measurements are shown in Figure 4.5, left (a). Three different concentrations of PE in the coating solution were tested (0, 0.1, 0.2 and 1.0 wt %). Activity was observed in the absence of PE, consistent with the confocal images discussed previously (see Figure 4.4). Moreover, enzyme activity increased with higher PE concentrations, reaching a maximum of 100 U/m^2 at 0.2 wt %. However, at 1.0 wt % PE, the immobilized activity decreases again to 50 U/m^2 . It is theorized that at higher PE concentrations, PEC primarily forms in the bulk solution rather than at the surface. This also leads to the formation of larger PEC particles that can be easily washed away during rinsing and activity measurements. Furthermore, a higher amount of desorbed enzyme was observed in membranes with a PE coating, indicating that a greater amount of enzyme is immobilized mainly through electrostatic interactions.

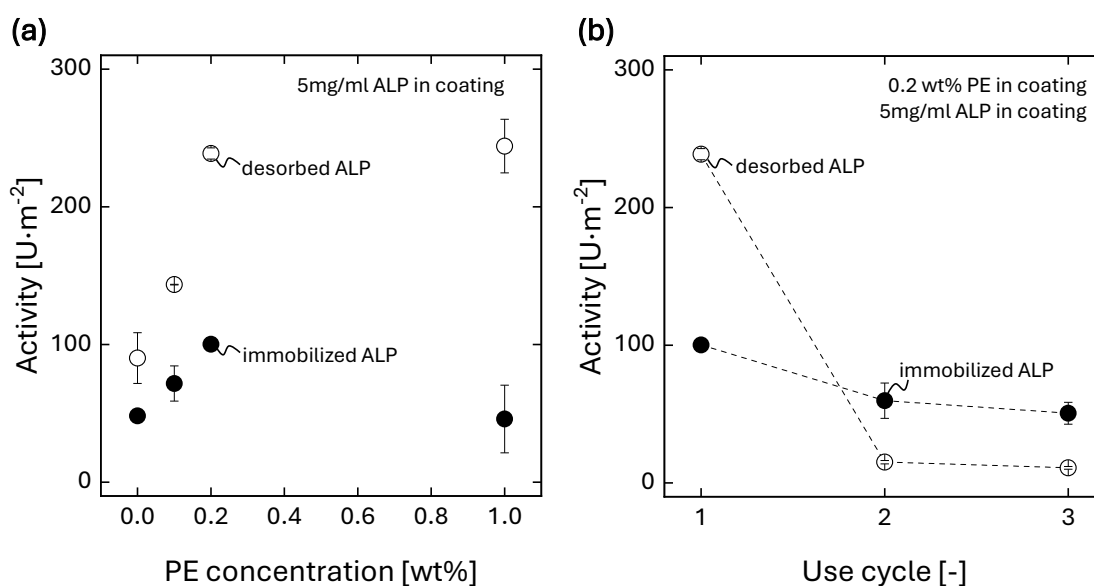


Figure 4.5: (a) Enzyme activity of PEC coated hollow fiber membranes via diffusive desalination. (b) Enzyme activity of ALP-PEC coated hollow fiber membranes over three reuse cycles. Membranes were coated using a 0.2 wt % PE solution and 5 mg/ml of dissolved ALP. Black markers denote the activity of the immobilized enzyme, white markers denote the activity of the enzyme that desorbed during the activity measurements.

To investigate the reusability of the coated HF, the membrane with the highest activity (0.2 wt %) was measured again for two more cycles. The results are depicted in Figure 4.5 (b). After the first measurement, the activity of the immobilized enzyme dropped from 100 U/m² to 60 and 50 U/m² after the second and third reuse cycles, respectively. On the other hand, the amount of desorbed enzyme decreases sharply after the first measurement cycle. However, at cycles 2 and 3, around 10 U/m² of desorbed enzyme is measured, which may partly account for the loss of immobilized activity between each cycle.

In general, the results obtained indicate that while enzyme immobilization within a PECs film is possible, there is a limitation on the loading capacity of the PEC film. One reason for this is the molecular size of the PEs, as the size of the used PEs range between 100-200 kDa, whereas the size of ALP is about 140-160 kDa. However, the actual spatial size of the ALP might significantly vary depending if the enzyme is in its native state, and the size of the PEs is also highly dependent on its interaction with the environment.

4.4 Conclusion

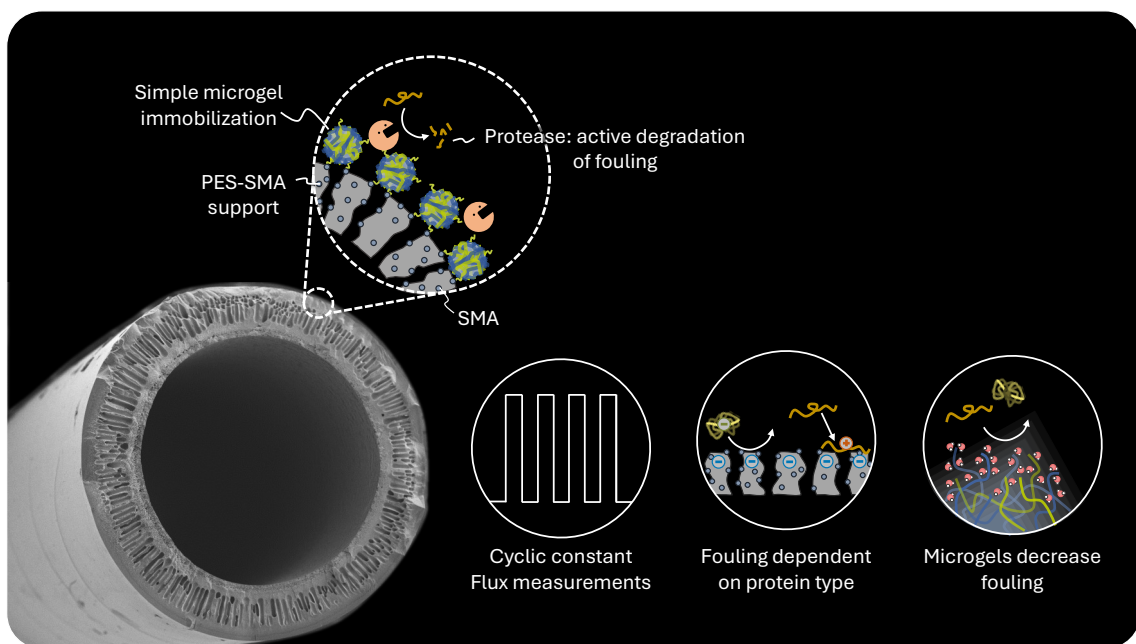
Porous and dense polyelectrolyte complex (PEC) layers evolve from single-step coating methods and serve as a versatile tool for the functionalization of hollow fiber membranes. The phase behavior of the polyelectrolyte system PSS:PDADMAC at various ionic strengths was utilized to build a selective barrier with nanofiltration properties or a polymer matrix for enzyme immobilization. Oppositely charged polyelectrolytes are dissolved simultaneously in water containing KBr. In contrast to the conventional layer-by-layer method, the polyelectrolyte coating is reduced to a single fabrication step.

For the fabrication of nanofiltration membranes, three methods are proposed. The resulting membranes exhibit MgCl_2 rejections of up to 79 % and permeabilities of 30 LMH/bar. For enzyme immobilization, the polyelectrolyte solution directly contains the enzyme ALP. FESEM images reveal the deposition of a polyelectrolyte layer, and confocal images show the enzyme ALP on the lumen surface. However, a reference experiment without polyelectrolytes also shows significant ALP immobilization. A polyelectrolyte concentration of 0.2 wt % leads to the highest activity of the immobilized enzyme of around 100 Up/m². Nevertheless, the activity of initially desorbed ALP is higher than the activity of the functionalized membrane. This indicates that the enzyme is mostly bound to the surface of the PEC layer via weak electrostatic interactions. After the initial desorption of weakly bound ALP, the enzymatic activity of the functionalized membrane stabilizes.

The presented single-step polyelectrolyte complex coating methods provide a potent alternative to the elaborate layer-by-layer coating procedure. By showcasing nanofiltration properties and enzymatic active membranes, a promising starting point is laid for further exploring and pushing the achievable membrane characteristics.

5

Low-fouling and self-cleaning hollow microgel-enzyme-coated membranes



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microgels on a blended PES-SMA support." *Journal of Membrane Science*, 2025, DOI: 10.1016/j.memsci.2025.124493

5.1 Introduction

Fouling is ubiquitous in membrane processes. It occurs when retained solutes accumulate on the membrane surface, leading to a decline in membrane performance over time. Regular mechanical cleaning methods, such as bubble scoring or backwashing, can effectively address reversible fouling [Pein2010]. However, irreversible fouling requires extensive chemical cleaning for its removal [Guo2012]. Although necessary, cleaning leads to prolonged downtime, increased maintenance labor, additional chemical costs, and a shortened membrane lifespan [Shi2014; Pein2010; Guo2012]. This has led to significant efforts to improve materials and process design to minimize fouling, particularly in water treatment, food, and pharmaceutical applications [Rana2010; Díez2020; Brug2009]. A major cause of irreversible fouling is the interaction between hydrophobic compounds - such as proteins, lipids, and natural organic matter- and hydrophobic polymers commonly used in membranes, such as polyethersulfone (PES) and polyvinylidene fluoride (PVDF). Enhancing membrane hydrophilicity is widely recognized as an effective strategy for improving fouling resistance [Huis2000; Rana2010].

Membrane hydrophilicity can be enhanced by coating with hydrophilic additives such as polyelectrolytes, polydopamine, nanoparticles, and zwitterionic polymers [Yan2020; Zhan2016; Rana2010]. Other materials that have recently gained attention as antifouling agents are microgels [Akso2019; Mait2022; Mait2023; Mish2022; Chen2013]. Microgels are 3D cross-linked hydrophilic polymer networks of colloidal size that can absorb significant amounts of water, resulting in a substantial increase in their mass and volume [Karg2019]. In addition, microgels can be tuned to respond to stimuli such as temperature and pH, making them helpful in controlling transport parameters during filtration [Bart2018; Bell2021]. However, like other hydrophilic additives, microgels can be easily washed away during operation [Wies2019; dGroot2015; Miya1990].

To ensure the long-term stability of a microgel coating, the layer should ideally be covalently attached to the membrane surface. This can be

achieved by cross-linking functional groups in the microgels and the membrane surface [Vito2017]. However, polymers often used in membrane fabrication, such as PES or PVDF, lack the required functional groups. Therefore, the membrane surface still requires some pre-treatment, which usually involves using harmful chemicals or time-consuming processes [Rana2010; Zhan2016].

An alternative to membrane pre-treatment is blending suitable copolymers in the membrane matrix that facilitate subsequent functionalization. For instance, the copolymer styrene maleic anhydride (SMA) can easily blend with polymers such as PES or PVDF to form porous membranes [Xian2012; Zhu2007]. SMA consists of a hydrophobic styrene section, which enables a stable anchor to the hydrophobic membrane matrix [Helm2024], and a maleic anhydride section, which enables subsequent functionalization due to its high reactivity towards nucleophiles [Jin2019]. Indeed, SMA enables the straightforward immobilization of polyelectrolytes and zwitterionic polymers to fabricate nanofiltration and low fouling membranes [Jin2020; Hu2023; Helm2024; Lin2021].

Moreover, the antifouling properties of membranes can be further enhanced by imparting membranes with the ability to self-clean [Vana2018; Schm2018; Schu2015; Schu2017]. This can be achieved by immobilizing digestive enzymes on the surface of the membrane. Indeed, a wide variety of enzymes is capable of breaking down the most common foulants. For instance, lipases can degrade oils and fats, amylases can break down long-chain carbohydrates, and proteases are responsible for breaking down proteins. In the case of proteases, it is best-practice to covalently attach the enzyme to the membrane to prevent self-hydrolysis [Vana2018]. Therefore, it is essential that the membrane also possesses adequate functional groups to which the enzymes can be attached [Joch2011].

Figure 5.1 conceptualizes the microgel-immobilization process explored in this work. Poly(N-isopropylacrylamide)-based microgels (pNIPAM) are synthesized with the co-monomer 2-aminoethyl methacrylate hydrochloride (AEMA) to introduce amine functionality. A blend of PES and SMA is used to fabricate the hollow fiber support. After membrane fabrication, the mem-

branes are immersed in a solution containing the microgels. In weak alkaline environments, the anhydride groups in the SMA react with the amine groups in the amine groups to form an imide bond. To demonstrate the antifouling properties of the modified membranes, we test the membranes in extensive fouling experiments ranging from static protein adsorption to cyclic constant flux filtration with different types of proteins. Additionally, the proteolytic enzyme trypsin is covalently attached to the membrane using a cross-linker. To evaluate the effectiveness of the enzyme immobilization, the membranes are fouled using a protein solution and then allowed to self-clean overnight. The permeability of the membrane is assessed to investigate the extent of the fouling/self-cleaning.

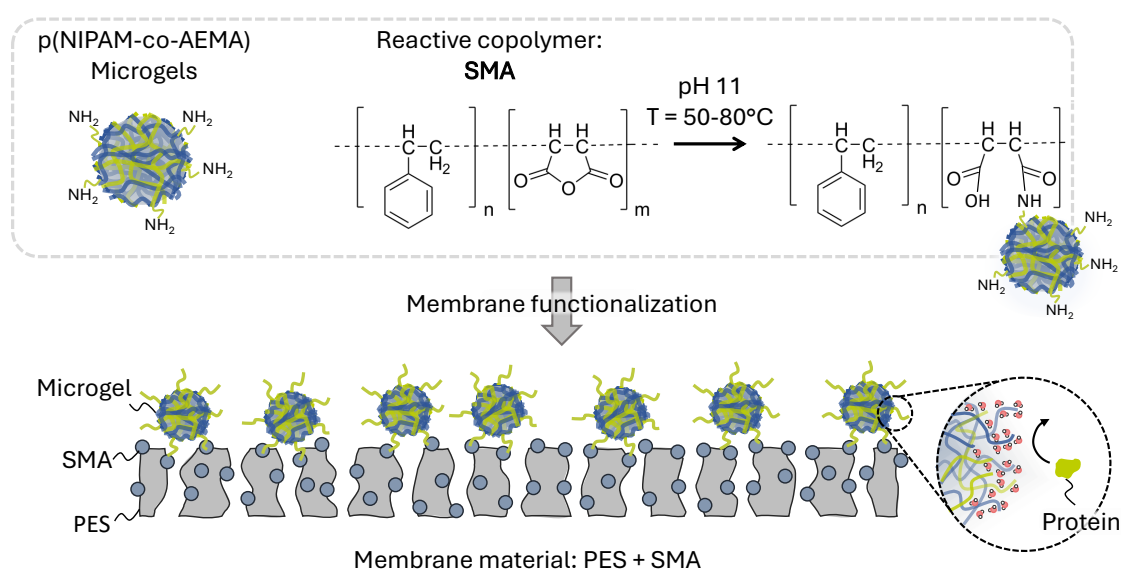


Figure 5.1: Chemical structure of the p(NIPAM-co-AEMA) microgels and the schematic representation of the microgel immobilization process.

5.2 Experimental

5.2.1 Materials

For the microgel synthesis, the monomer *N*-isopropylacrylamide (NIPAM) (>98%) and the initiator 2,2-Azobis(2-methylpropionamidine) dihydrochloride (AMPA) were purchased from TCI. The cross-linker *N,N'*-methylenebis(acrylamide) (BIS) and the surfactant cetyltrimethylammonium bromide (CTAB) were obtained from Sigma-Aldrich. The co-monomer AEMA was supplied from Polyscience. Before use, NIPAM was recrystallised in hexane (99%, VWR) and dried under vacuum. To purify the microgel solutions, 14 kDa dialysis membranes were purchased from Carl Roth.

For hollow fiber fabrication, PES (Ultrason E6020 P) and polyvinylpyrrolidone K17 (PVP K17) ($M_W \sim 10$ kDa) were supplied by BASF. The SMA-copolymer XIRAN® SZ30010 ($MW \sim 10$ kDa), was kindly provided by Aurorium. Polyvinylpyrrolidone K30 ($M_W \sim 40$ kDa) and polyethyleneglycol ($M_W \sim 400$ Da) were purchased from Carl Roth. The solvent *N*-methyl-2-pyrrolidone (NMP) (99%) was obtained from Fisher Scientific.

For the fouling experiments, the proteins bovine serum albumin (BSA) (heat shocked fractioned, >98%), albumin–fluorescein isothiocyanate conjugate (f-BSA), and gelatin from porcine skin (gel strength 300, type A) were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS) was obtained from TH Geyer.

Furthermore, trypsin from porcine pancreas, tris(hydroxymethyl) aminomethane (Tris), calcium chloride anhydrous (CaCl_2) (>99.99%) and sodium hydroxide (NaOH) (>98%) were purchased from Sigma-Aldrich.

5.2.2 Hollow fiber spinning and module construction

Composition of the polymer and bore solutions are listed in Table 5.1. The polymer solution was prepared by combining all listed components in a heated glass reactor at 60 °C and mixing with a mechanical stirrer until fully dissolved. The solution is then transferred to the spinning set-up to allow

the solution to degas overnight. The bore solution was prepared by mixing all listed components in a glass bottle with a magnetic stirrer.

Table 5.1: Composition of polymer and bore solutions used for membrane fabrication.

Solution	PES [wt.%]	SMA [wt.%]	PVP K17 [wt.%]	PVP K30 [wt.%]	PEG 400 [wt.%]	NMP [wt.%]	DI Water [wt.%]
PES-SMA	17.5	2	16	4	4	56.5	-
PES	17.5	-	16	4	4	58.5	-
Bore	-	-	-	-	10	60	30

Hollow fiber membranes were fabricated via a dry-jet wet phase inversion spinning technique. In this process, the polymer solution is extruded through a spinneret alongside the bore solution. Phase inversion begins upon contact with the bore solution, with partial coagulation occurring in the air gap. The hollow fibers then pass through a coagulation bath, where they solidify completely. Finally, the fibers pass through a first rinsing bath and are collected on a second rinsing bath. More detailed spinning parameters are listed in Table 5.2. After spinning, the fibers were immersed in DI water for 48 h to remove the remaining NMP, with an exchange of water conducted after 24 h. Then, the membranes are immersed in a 60 °C water bath for 6 h to wash out PVP from the membranes. Finally, the membranes are then placed in a bath of 50 w% glycerol/water overnight and are subsequently dried.

4-end outside-in hollow fiber modules with 58 fibers and an active length of 10 cm were manufactured using a potting centrifuge (RotaMini, Me-Sep, Poland). The resulting active membrane area was approximately 195 cm². Before use, the modules were flushed with DI-water and filled from the inside with water using a syringe. If not in use, the modules were stored wet at room temperature.

Table 5.2: Spinning parameters used for membrane fabrication

Parameter	Unit	Value
Temperature bore	°C	RT
Temperature polymer	°C	30
Temperature coagulation bath	°C	40
Temperature spinneret	°C	RT
Flow rate polymer	mL min ⁻¹	3.6
Flow rate bore	mL min ⁻¹	0.8
Air gap height	cm	16.5
Pulling wheel speed	m min ⁻¹	6.8

5.2.3 Microgel synthesis

p(NIPAM-co-AEMA) microgels were synthesized via precipitation polymerization following the method previously described [dS Me2024]. In short, the reaction was carried out in a round-bottom flask at 80 °C under stirring at 300 rpm in a nitrogen atmosphere. The specific components were dissolved in a given volume of DI-water in three separate vials. A detailed composition is listed in Table 5.3. The reaction starts by adding the initiator AMPA (vial 2) to a mixture of NIPAM, BIS, and the surfactant CTAB (vial 1). Two minutes into the reaction, AEMA and additional AMPA are injected (vial 3). After a total reaction time of 25 min, the polymerization is stopped by cooling the flask in ice water in the presence of oxygen. Finally, the microgels were purified using a dialysis tube.

5.2.4 Membrane modification

Microgel immobilization

For microgel immobilization, a microgel solution with a concentration of 0.5 mg/ml and pH 11 is obtained by dilution with diluted sodium hydroxide (NaOH). The concentration of the initial microgel solution was previously determined by freeze-drying a defined volume of the solution and weighting the amount of dried microgels. For the coating, the shell side of the hollow

Table 5.3: Recipe for the p(NIPAM-co-AEMA) microgel synthesis.

Vial	Component	Amount mg	Volume ml
1	NIPAM	220.8	-
	BIS	13.5	-
	CTAB	5.8	-
	DI-water	-	24
2	AMPA	10.6	-
	DI-water	-	3.3
3	AEMA	321.2	-
	AMPA	26.5	-
	DI-water	-	2.7

fiber modules is flushed and filled with the microgel solution. The filled modules are then submerged in a temperature-controlled water bath for 5 min at 60 or 80 °C. After the coating process, the modules are thoroughly rinsed and stored in DI-water.

Enzyme immobilization

Prior to enzyme immobilization, the membranes were thoroughly rinsed with DI-water. The modules were then filled with a 2.5 wt % GA dissolved in a carbonate buffer solution (pH 10, 50mM). The membranes were pre-activated with the cross-linker for 2 h at room temperature under mild shaking. Afterward, the GA solution was removed and the membrane was rinsed three times by flushing 500 ml DI-water across the module.

After rinsing, the modules are filled with a 10 mg/ml trypsin dissolved in a Tris buffer solution (25mM, pH 8.5, 10mM CaCl₂). Enzyme immobilization was conducted at room temperature under mild shaking for 2 h. Afterward, the enzyme solution was removed and the membrane was rinsed three times by flushing 500 ml DI-water across the module. Finally, the modules were filled with the Tris buffer and were left under mild shaking over night at room temperature. On the next day, the buffer was exchanged by

fresh Tris buffer. The modules were stored at 5 °C in Tris buffer when not in use. Table 5.4 summarizes all types of membranes and modifications used in this study.

Table 5.4: Overview of membranes characterized in this study

Membrane	Base Polymer	Modification	Enzyme immobilization
P-0	PES	-	-
PS-0	PES-SMA	-	-
MG-60C	PES-SMA	0.5 mg mL ⁻¹ microgel at 60 °C	-
MG-80C	PES-SMA	0.5 mg mL ⁻¹ microgel at 80 °C	-
P-0-T	PES	-	10 mg/ml trypsin
PS-0-T	PES-SMA	-	10 mg/ml trypsin
MG-60C-T	PES-SMA	0.5 mg mL ⁻¹ microgel at 60 °C	10 mg/ml trypsin
MG-80C-T	PES-SMA	0.5 mg mL ⁻¹ microgel at 80 °C	10 mg/ml trypsin

5.2.5 Membrane characterization

Pure Water Permeability

The pure water permeability (PWP) of the modules is measured before and after coating, and in between fouling experiments. First, water is permeated in dead-end from the shell to the lumen of the fiber at 1 bar transmembrane pressure (TMP) for at least 20 min. A scale continuously records the weight of the permeate produced. To ensure the validity of the results, the PWP is measured twice for each module.

Field emission scanning electron microscopy

FESEM imaging was used to analyze the morphology of membranes. Samples were fractured in liquid nitrogen to obtain cross-sectional images. Surface images were obtained by cutting the membrane samples lengthwise. A Hitachi SU5000 was used to obtain the images.

Zeta Potential

The surface zeta potential of flat sheet membranes was determined by a SurPASS analyzer (Anton Paar, Graz Austria) in a pH range of 2-9 with a 1 mM KCl electrolyte solution. The pH is automatically adjusted by two solutions of 0.1 M HCl and 0.1 M NaOH.

5.2.6 Fouling analysis

Three types of fouling experiments were conducted to investigate the antifouling properties of different membranes: Flux stepping, cyclic fouling at constant flux, and static fouling experiments. An automatized filtration set-up was used for the flux stepping and the cyclic fouling experiments (OSMO Poseidon, Demcon convergence, The Netherlands). A qualitative representation of the sequence of the dynamic filtration experiments is showcased in Figure 5.2.

Flux stepping fouling

To better characterize the antifouling behavior of membranes, examining them at a permeate flux at which severe fouling occurs is necessary. This occurs when permeate fluxes exceed the threshold flux J_{th} . Therefore, J_{th} was determined for all modified and unmodified membranes by conducting flux stepping experiments. Before filtration, the membranes were flushed with DI-water for 5 min to ensure no air bubbles were present in the module. Afterward, nine constant flux filtration cycles were conducted using a model fouling solution (BSA, 0.5 g L^{-1} in PBS pH 7.4). The flux steps start from 10 LMH and increase 5 LMH after each step. Each step consists of an initial flushing with the feed solution for 4 min to fill the module and the tubing with the protein solution. Then, the permeate flux is set to the desired flux for a duration of 25 min and is followed by a backwashing step at 50 LMH for 10 min with DI water. The modules are operated outside-in in cross-flow mode. The feed flow rate during permeation was set at 4 kg h^{-1} .

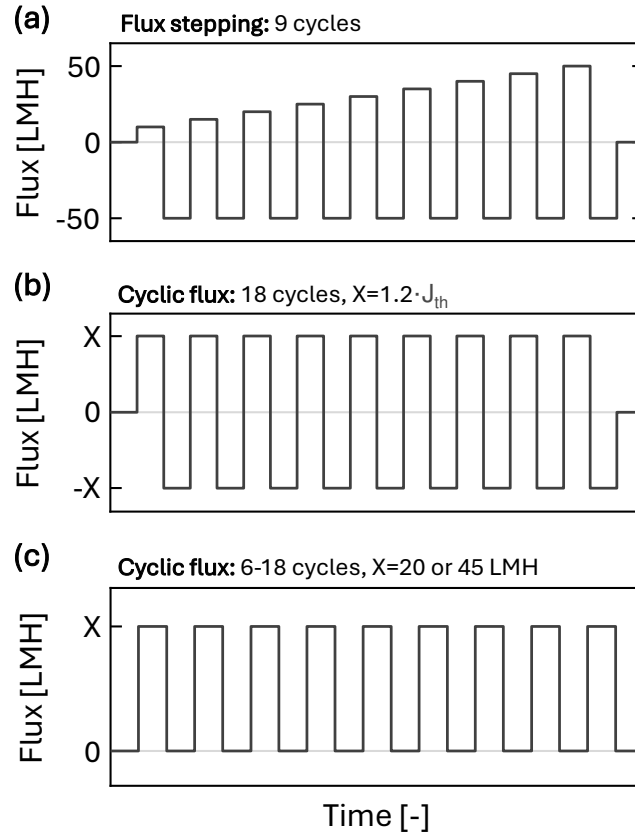


Figure 5.2: (a) Flux stepping filtration method, (b) cyclic filtration at constant flux with backwashing method, and (c) cyclic filtration at constant flux with relaxation method

Cyclic fouling at $1.2 \cdot J_{thres}$ with backwashing

Similar to the flux stepping experiments, the sequence begins by flushing with DI-water for 5 min. Then, each filtration step begins by flushing with the model solution (BSA, 0.5 g L^{-1} in PBS pH 7.4) for 4 min after which the permeate flux is set to 1.2 times J_{th} of the respective membrane. The total amount of permeate for each cycle is 180 g. This results in different step lengths for the different threshold fluxes. Each permeation step is followed by a backwashing step with DI water at a backwashing flux of 1.2 times the J_{th} . In each backwashing step, 90 g of DI-water permeates through the membrane. In total, 18 filtration cycles are conducted for each membrane. During all steps, the modules are operated outside-in in cross-flow mode. The feed flow rate during permeation is set at 4 kg h^{-1} .

Cyclic fouling at constant flux with relaxation

These experiments were conducted in a manner similar to that previously described. However, the backwashing steps in the sequence are replaced by relaxation steps. The model fouling solution consists of either dissolved BSA or gelatin type A (0.5 g L^{-1} in PBS pH 7.4). Filtration steps are conducted at 45 LMH and 20 LMH for BSA and gelatin, respectively. The duration of the permeation is set to produce 180 g of permeate. The relaxation steps are equivalent to flushing the module with DI water at a feed flux of 4 kg h^{-1} for 10 min. When BSA is used as the foulant, 18 filtration cycles are conducted. When gelatin is used, only 8 cycles are conducted. The modules are operated outside-in in cross-flow mode. The feed flow rate during permeation is set at 4 kg h^{-1} .

Evaluation of static protein adsorption via fluorescence microscopy

Static fouling measurements were performed to qualitatively evaluate the antifouling properties of microgel-coated membranes. Fluorescein isothiocyanate-labeled bovine serum albumin (f-BSA) was used as the reference foulant and adsorbed to the membranes.

FS membranes with a diameter of 15 mm were used in a 24-well plate. Before the adsorption, the membranes were thoroughly rinsed with 2 mL PBS buffer (pH 7.4). Then, 2 mL f-BSA solution with a concentration of 50 mg mL^{-1} in PBS buffer were added to each well. The adsorption took place for 3 h. The well plate was wrapped in aluminum foil to protect the fluorescein marker from bleaching. Subsequently, the membranes were again rinsed with 2 mL PBS buffer as previously described. The membranes were placed between two microscope slides, and any air bubbles were removed. The samples were again wrapped in aluminum foil and stored at 5°C until further analysis. The fluorescein marker on the BSA enables the evaluation with a fluorescence microscope (BZ-X810, Keyence Deutschland GmbH, Germany). All images were taken with the same exposure settings.

5.2.7 Evaluation of the self-cleaning properties

To evaluate the self-cleaning properties of the enzyme-modified membranes, the membrane modules were initially fouled by flushing a gelatin solution (0.5 mg mL^{-1} , PBS buffer, pH 7.4) in cross-flow at 1 bar TMP until 100 mL permeate were produced. After fouling, the membranes were rinsed with DI-water until 50 mL permeate was produced. The PWP of the membrane modules was measured before and after fouling to evaluate the degree of fouling. To initiate the enzymatic self-cleaning, the modules were filled with a Tris buffer solution (25mM, pH 8.5, 10mM CaCl_2) and left under mild shaking for 16 h. Afterward, the Tris buffer was removed, and the modules were rinsed with DI-water in cross-flow at 1 bar TMP until 50 ml permeate was produced. The PWP was subsequently measured. The modules were stored in Tris buffer at 5°C .

5.3 Results and Discussion

5.3.1 Membrane fabrication and modification

Hollow fiber membrane fabrication was achieved by a dry-wet jet spinning process. A polymer solution consisting of PES, SMA, and other pore-forming additives was extruded in a water coagulation bath. A bore solution composed primarily of NMP was used to achieve an outside-in geometry. For the exact composition of both polymer and bore solution and the spinning parameters, see Section 5.2.2. After fabrication, membranes were modified according to parameters listed in Section 5.2.4. SEM images of the hollow fibers and the effect of the modification on the membrane surface are showcased in Figure 5.3.

The cross-sectional images of the membranes (a-c) show an asymmetric structure with a dense layer on the shell side, an open-porous layer on the lumen side, and finger-like macrovoids on the midsection of the fiber. The surface of the selective shell layer of the unmodified PS-0 membrane (d) shows the presence of small pores. In both MG-60C and MG-80C, a deposition of spherical aggregates completely covers the surface pores. These particles, with a diameter of approximately 120 μm , appear to correspond to the microgels. No significant differences are observed between the coating temperatures (see Figure 5.3 (e) and (f)).

5.3.2 Surface properties of microgel coated membranes

As discussed in Section 5.3.1, an apparent deposition of a microgel layer is observed after the coating. To elucidate the effect of the microgel layer on the surface properties of the membrane, zeta potential, and PWP measurements were conducted on unmodified PES-SMA membranes (PS-0), microgel-modified PES-SMA membranes (MG-60C and MG-80C). As further reference, also unmodified PES membranes (P-0) are evaluated.

Figure 5.4 (a) presents the zeta potential of flat-sheet coated membranes as a function of pH. P-0 membranes present a negative zeta potential, which is expected from unmodified PES membranes [Helm2024]. By adding SMA

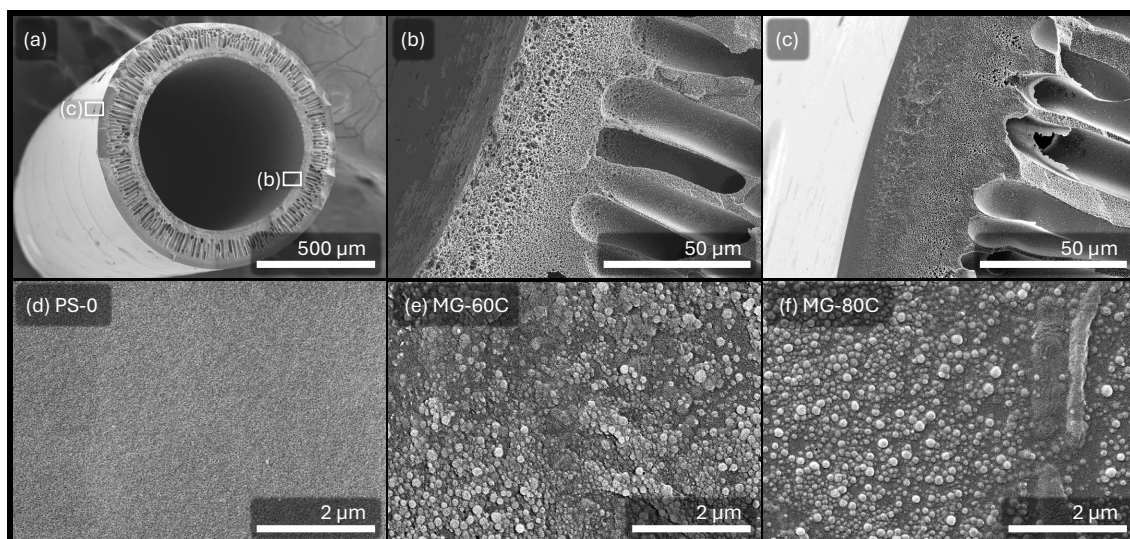


Figure 5.3: Cross-sectional SEM images of the PS-0 hollow fiber membranes, and images of the shell surface before and after microgel immobilization.

as copolymer (PS-0), membranes become more negatively charged, which is reflected by a more negative zeta potential, especially above a pH of 4. In both MG-60C and MG-80C, a deposition of spherical aggregates is observed to cover the surface pores completely. The particles, with a diameter of approximately 120 μm, appear to correspond to the microgels. No significant differences are observed between the coating temperatures. Notably, MG-80C displays a more positive zeta potential compared to MG-60C, which suggests a higher amount of immobilized microgels on the MG-80C surface.

PWP measurements (Figure 5.4 (b)) further confirm the deposition of a microgel layer on the membrane surface. PS-0 membrane has a PWP of around 120 LMH/bar. After the MG coating, PWP is reduced by approximately 60 % and 80 % in the case of MG-60C and MG-80C membranes, respectively. The more significant decrease in PWP observed for MG-80C suggests a greater amount of immobilized microgels, consistent with the zeta potential results. P-0 has a similar PWP to PS-0, which can be attributed to the similar compositions of the polymer solutions.

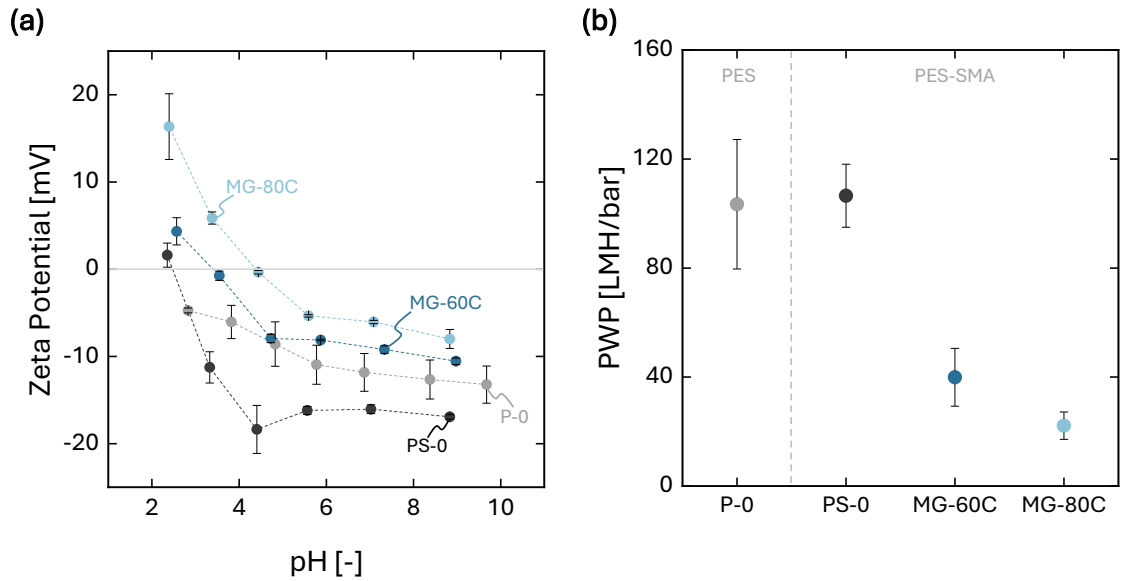


Figure 5.4: Effect of microgel immobilization on (a) the zeta potential of the membranes, and (b) on the pure water permeability (PWP). As reference, a membrane fabricated without SMA as additive (P-0) is included.

5.3.3 Threshold flux determination

Fouling and the fouling rate are directly dependent on the permeate flux. This correlation is described by the critical flux (J_{crit}) and the threshold flux (J_{th}). J_{crit} is the permeate flux below which no fouling can be observed [Fiel1995]. J_{th} is the flux below which the fouling rate is constant and above which the fouling rate increases significantly [Fiel2011; Mill2014]. Both J_{crit} and J_{th} depend on membrane properties, flow conditions, and other process variables [Fiel2011].

Flux stepping experiments are widely employed to determine both J_{crit} and J_{th} [Drew2010]. In these experiments, the flux is increased step by step and held constant for a fixed period of time at each step. The step length, the step height, the feed properties and the flow conditions are factors that influence the fouling behavior. Therefore, the flux stepping procedure itself affects the threshold fluxes or the critical fluxes that are obtained as a result of the experiment [Clec2003]. In order to reduce the effects of fouling from previous steps, cleaning steps can be employed between the fouling steps [vdMare2009; Drew2010]. However, due to the long filtration times needed to determine J_{crit} [Fiel2011; Drew2010], flux stepping experiments are better

suited to identifying J_{th} [Mill2014; Drew2010].

The membranes evaluated in this study differ significantly in their intrinsic filtration resistance and surface charge. This is expected to result in different fouling tendencies under similar filtration conditions. In order to accurately assess whether the microgel coating improves the antifouling properties, membranes should be tested under conditions where fouling has a significant impact. Therefore, membranes were evaluated at fluxes above their threshold flux (J_{th}).

Flux stepping experiments were performed to determine J_{th} . In these experiments the flux was gradually increased in 5 LMH steps from 10 LMH to 50 LMH using BSA as the model foulant. Each filtration step was followed by a backwashing step with deionised water. The TMP evolution over time was recorded for each membrane type, providing data from which three values were extracted to determine J_{th} : the average TMP at each step (TMP_{avg}), the change in TMP between steps (ΔTMP), and the TMP gradient over time ($d(TMP)/d(t)$). These values were plotted against the flux, and J_{th} was determined according to the method presented by Miller et al. [Mill2014].

The results of the flux stepping experiments are shown in Figure 5.5. Three distinct domains were observed for P-0, PS-0 and MG-60C: (1) a domain with no significant increase in TMP (10 LMH - 20 LMH); (2) a domain where TMP reaches a plateau (25 - 35 LMH); and (3) a domain with no plateau and a sharp increase in TMP during each filtration step (40 - 50 LMH). Domains 2 and 3 appear to start at a slightly lower flux level for MG-60C. However, the presence of domain 1 for P-0, PS-0, and MG-60C suggests that their J_{th} values are within the flux range tested. For the MG-80C membrane, only domains 2 and 3 were observed, allowing for the determination of J_{th} , although with less precision. Unfortunately, the minimum flux setting for the membrane module used is 10 LMH, which limits further accuracy of J_{th} for the MG-80C.

Table 5.5 lists the resulting J_{th} for the methods using (a) TMP_{avg} , (b) ΔTMP , (c) $d(TMP)/d(t)$. Only the values of J_{th} determined by TMP_{avg} are considered, as this method gives the highest value of J_{th} . Subsequent experiments use the rounded values of $1.2 \cdot J_{th}$ calculated using $J_{th,a}$.

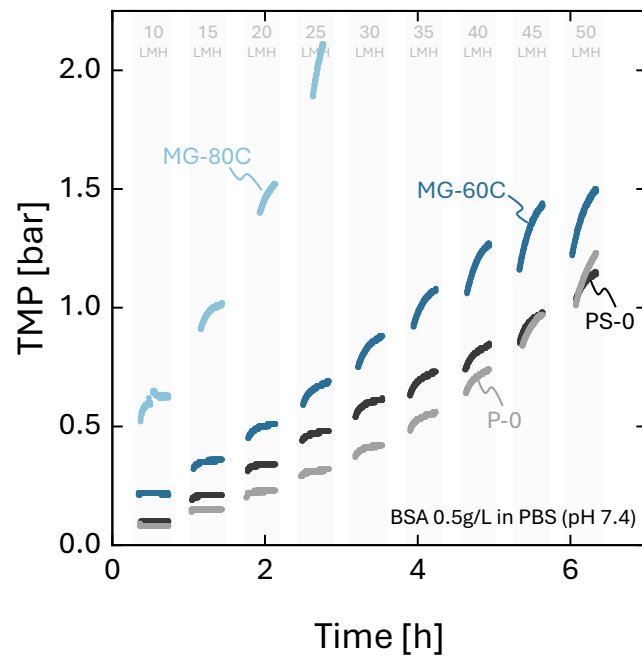


Figure 5.5: Flux stepping experiments with unmodified PES and PES-SMA hollow fiber membranes, and modified PES-SMA hollow fiber membranes with 0.5 g L^{-1} BSA as the model foulant. The flux was increased from 10 LMH to 50 LMH in 5 LMH steps. Each step had a duration of 25 min and was followed by a 10 min backwashing step.

Table 5.5: Resulting J_{th} of the different membranes evaluated.

Membrane	$J_{th,a}$ LMH	$J_{th,b}$ LMH	$J_{th,c}$ LMH	$1.2 \cdot J_{th,a}$ LMH
P-0	33	25	20	40
PS-0	28.3	25	26	34
MG-60C	22.1	20	20	27
MG-80C	16.6	15	15	20

5.3.4 Cyclic fouling at constant flux with BSA

To assess the effect of membrane modification on antifouling properties, constant flux experiments were performed at $1.2 \cdot J_{th}$ for each membrane (see Table 5.5) and at 45 LMH/bar. Each experiment consisted of 16 filtration cycles. For cycles with a flux set to $1.2 \cdot J_{th}$, backwashing at $1.2 \cdot J_{th}$ was

performed between cycles. Only a relaxation step was conducted between each cycle for cyclic experiments with a flux of 45 LMH. See Section 5.2.6 for a more detailed method description. Note that the membranes were rinsed with BSA solution before each filtration step to ensure complete filling of the module with the protein solution. Due to pressure losses in the module, a low TMP is initially established, leading to unregulated permeation, during which fouling can begin. This means that the permeability displayed at the beginning of each step may not accurately reflect the actual permeability of the module after backwashing/relaxation. Therefore, the relative loss of pure water permeability was assessed every six cycles to evaluate irreversible fouling. The results of the experiments are shown in Figure 5.6, with (a) and (b) for $1.2 \cdot J_{th}$, (c) and (d) for 45 LMH.

The cyclic fouling experiments at $1.2 \cdot J_{th}$ demonstrate reversible fouling in each filtration step for PS-0, MG-60C, and MG-80C membranes. Notably, PS-0 and MG-60C gradually increased their final permeability with each cycle. On the other hand, P-0 membranes do show a gradual decrease in final permeability, which also correlates with the trend observed in the PWP measurements. Conversely, PS-0, MG-80C, and MG-60C do not show a significant decrease in PWP over the 18 cycles, indicating minimal to no irreversible fouling.

Interestingly, the gradual increase in final permeability observed in PS-0 and MG-60C may suggest that less BSA is adsorbed on the membrane surface over successive cycles since the baseline PWP is not significantly affected. This may be due to increased electrostatic repulsion of the negatively charged BSA as the membrane surface becomes more negatively charged. Indeed, SMA may undergo further ring opening under physiological pH conditions [Pomp2005]. Although the SMA in MG-60C membranes was initially activated by immersion in diluted NaOH at 60 °C, further hydrolysis in PBS buffer could occur. In the case of MG-80C membranes, the thicker microgel layer counteracts this effect (see Section 5.3.2.

At the higher flux of 45 LMH, membranes behaved similarly as previously described. The final permeability of P-0 gradually decreases after each subsequent cycle, indicating some degree of irreversible fouling. This is also

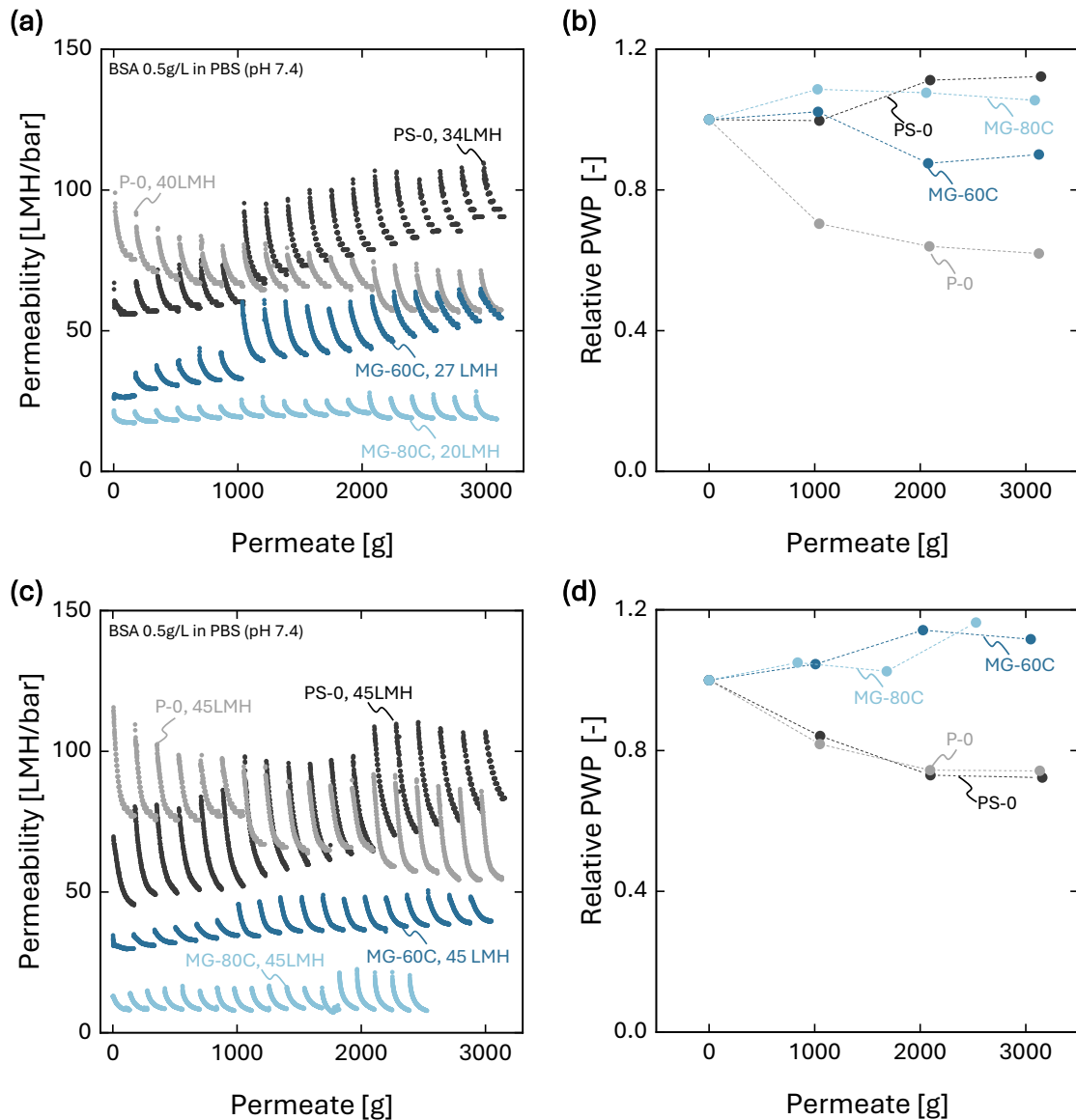


Figure 5.6: Fouling behavior of unmodified and modified hollow fiber membranes after 18 cycles of 0.5 g L^{-1} BSA filtration at constant flux. Reversible fouling was measured as a function of the decline of permeability over the produced permeate. Irreversible fouling was characterized by the change in the pure water permeability of the membranes in between 6 cycles. (a) Fouling experiments conducted at $1.2 \cdot J_{th}$ with backwashing, and (b) the corresponding pure water permeability of membranes in-between cycles. (c) Fouling experiments conducted at 45 LMH with relaxation steps in-between cycles, and (d) the corresponding pure water permeability.

confirmed by the decrease in PWP between cycles. In the case of PES-SMA membranes, both PS-0 and MG-60C showed gradual increases in final permeability, with the increase being more pronounced in PS-0. The MG-80C

membrane, however, displayed a consistent permeability profile across the 18 cycles, though it produced less permeate overall due to challenges in achieving the set flux. Due to the high TMP required in MG-80C, the device struggled to reach 45 LMH reducing the actual time operated at the target flux. Pure water permeability measurements confirmed no irreversible fouling in MG-60C and MG-80C, while PS-0 exhibited a decrease in pure water permeability, suggesting some accumulation of irreversible fouling.

5.3.5 Evaluation of static BSA adsorption

Static adsorption experiments with fluorescent-labeled BSA (f-BSA) were conducted on flat sheet membranes to corroborate the cyclic fouling experiments. Static adsorption was achieved by immersing membrane samples in a f-BSA solution under continuous shaking. Afterward, the membranes were washed with buffer solution, and the degree of adsorption was qualitatively evaluated according to the intensity of the fluorescent signal captured by a fluorescence microscope. A higher fluorescent signal is indicative of more significant BSA adsorption. The results are presented in Figure 5.7.

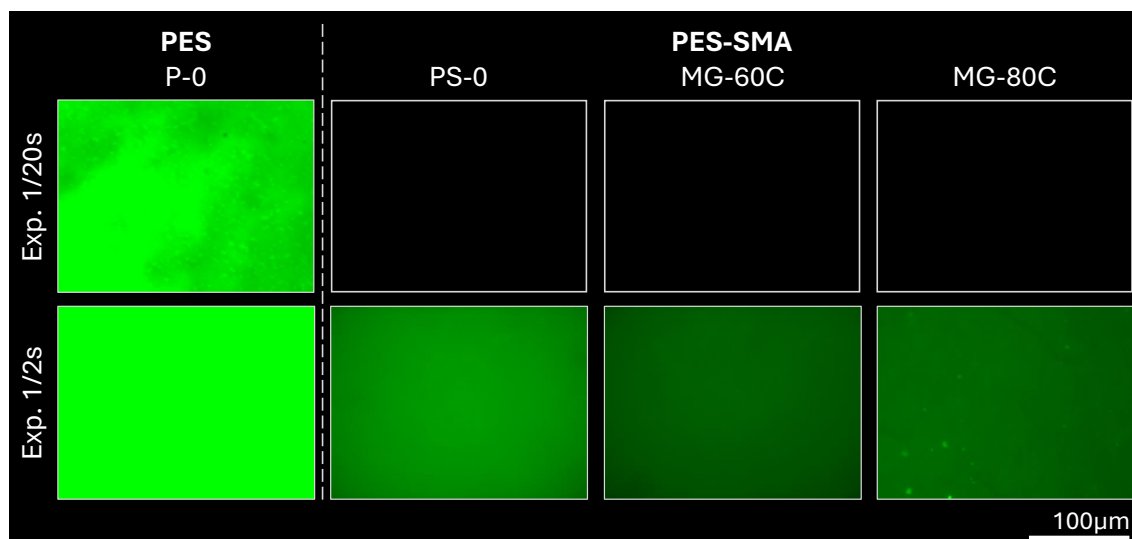


Figure 5.7: Static BSA adsorption on unmodified and modified PES-SMA flat sheet membranes. An unmodified PES membrane is used as reference. Two light exposure times (1/20 s and 1/2 s) are used to demonstrate the significantly lower adsorption of all PES-SMA membranes compared with membranes prepared only with PES. At the longer exposure time, a slightly higher protein adsorption can be observed for the PS-0 membrane compared with other PES-SMA membranes.

All PES-SMA membranes show no coloration at shorter exposure times (1/20 s). This is in stark contrast to the bright green of the PES membrane. At a longer exposure time (1/2 s), a slightly lighter color can be seen for the PS-0 membrane compared to MG-60C and MG-80C, which may indicate a higher fouling tendency of the unmodified PES-SMA membrane. However, from the overall results obtained, it can only be stated with certainty that all PES-SMA membranes, regardless of their modification, have a low affinity for BSA. In fact, a low BSA affinity for PES-SMA blended membranes has already been observed in the literature [Helm2024]. Furthermore, these results are also consistent with the dynamic fouling experiments. In the case of PS-0, these results are attributed to the electrostatic repulsion of the negatively charged BSA, which has an isoelectric point (IEP) between 4.7 and 4.1 [Jach2008]. In the case of MG-60C and MG-80C, it remains unclear whether the immobilized microgel layer may also enhance this effect by increasing the hydration layer on the membrane surface.

5.3.6 Cyclic fouling at constant flux with gelatin

Cyclic filtration experiments with BSA showed that microgel-modified membranes have better antifouling properties, especially at higher fluxes. However, it is not clear whether this low affinity for BSA is due to electrostatic repulsion between the protein and the membrane or whether this is due to a robust hydration layer formed by the microgel coating. Additional experiments were therefore carried out using gelatin type A. Gelatin type A, with an IEP between pH 7-8 [Bäth2023], is expected to be neutral or slightly positive charged under the experimental conditions used (PBS buffer, pH 7.4).

The fouling experiments were carried out as previously described, using six cyclic filtration cycles at constant flux with a gelatin solution (0.5 g L^{-1} in PBS buffer). Between cycles, relaxation steps were performed with water. Initially, a flux of 45 LMH was selected, resulting in a complete loss of permeability for a PS-0 membrane module during the first cycle. Therefore, a lower flux of 20 LMH was used. The results are shown in Figure 5.8.

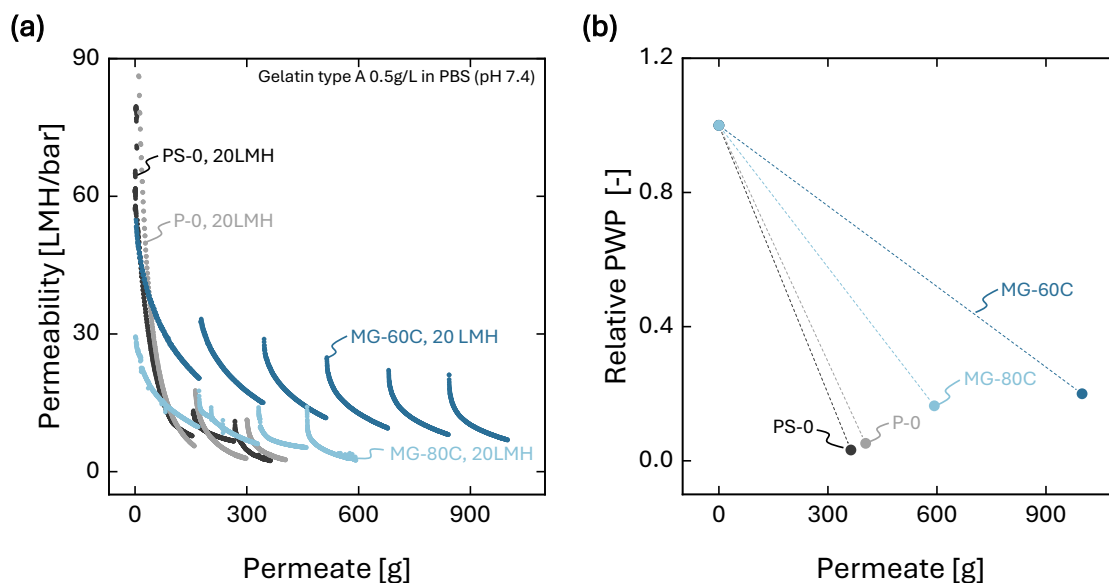


Figure 5.8: Fouling behavior of unmodified and modified hollow fiber membranes after 6 cycles of 0.5 g L^{-1} Gelatin filtration at 20 LMH. (a) Permeability decline as a function of the produced permeate. (b) Relative pure water permeability before and after the fouling experiments.

In general, more fouling was observed with gelatin compared to BSA, which is expected as protein fouling peaks near the IEP of the protein [Ang2007; Ma2016; She2009]. Proteins are most hydrophobic near their IEP, increasing adsorption to membrane surfaces via hydrophobic interactions [Huis2000]. Furthermore, after the formation of a protein monolayer, little to no electrostatic repulsion between proteins can exacerbate further adsorption [Ang2007].

Differences in fouling behavior were observed between the membranes. Both microgel-coated membranes experienced fouling but with a less severe permeability decrease than P-0 and PS-0. MG-60C completed all six cycles before reaching a TMP pressure limit of 5 bar. In contrast, experiments with P-0, PS-0, and MG-80C were terminated early due to the high TMP reached. After the first cycle and relaxation step, both MG-60C and MG-80C lost approximately one-third of their initial permeability; PS-0 lost approximately 90 % permeability in the first cycle with no significant recovery after relaxation. Subsequent cycles showed less severe fouling for MG-60C and MG-80C, as evidenced by significant permeability recovery after relaxation.

Furthermore, no direct relationship between surface charge and fouling was observed. This is evidenced by the similar performance of P-0 and PS-0 despite the more negative zeta potential of PS-0 (see Figure 5.4 (a)). Moreover, MG-60C also outperforms MG-80G despite the more positive zeta potential of MG-80G (see Figure 5.4 (a)). However, the difference between MG-60C and MG-80C may be due to the lower initial permeability of MG-80C. In the case of P-0 and PS-0, the significant decrease in permeability is attributed to a more hydrophobic character. Indeed, Huisman et al. previously demonstrated that stronger hydrophobic interactions between membranes and proteins are responsible for a more significant initial permeability decrease in constant pressure filtration. In contrast, electrostatic interactions did not correlate with permeability decrease at any stage [Huis2000]. Thus, the presented results demonstrate that the improved antifouling properties of the microgel are primarily due to increased water adsorption rather than electrostatic repulsion alone.

5.3.7 Enzymatic self-cleaning

To evaluate the effectiveness of the enzyme immobilization on the fouling removal, membranes were fouled following a constant TMP filtration (see Section 5.2.7). After fouling, the membranes were filled with tris buffer (25mM, pH 8.5, 10mM CaCl₂) and left under mild shaking over night at room temperature to allow for the enzyme to react. On the next day, the PWP of the membranes was measured again. Figure 5.9 showcases the results of the experiments. The PWP is given as the relative PWP calculated from the PWP measured before fouling. Moreover, since enzyme immobilization also leads to a decrease in PWP, the PWP values before and after trypsin immobilization are summarized in Table 5.6.

In the case of the unmodified PES membrane P-0-T, PWP decreased to approximately 20 %. Surprisingly, the membrane recovered 70 % of its PWP following self-cleaning. Moreover, after six days in storage, the membrane recovered further to 80 % of its initial PWP. A similar trend was observed during the second fouling cycle. These results suggest the presence of

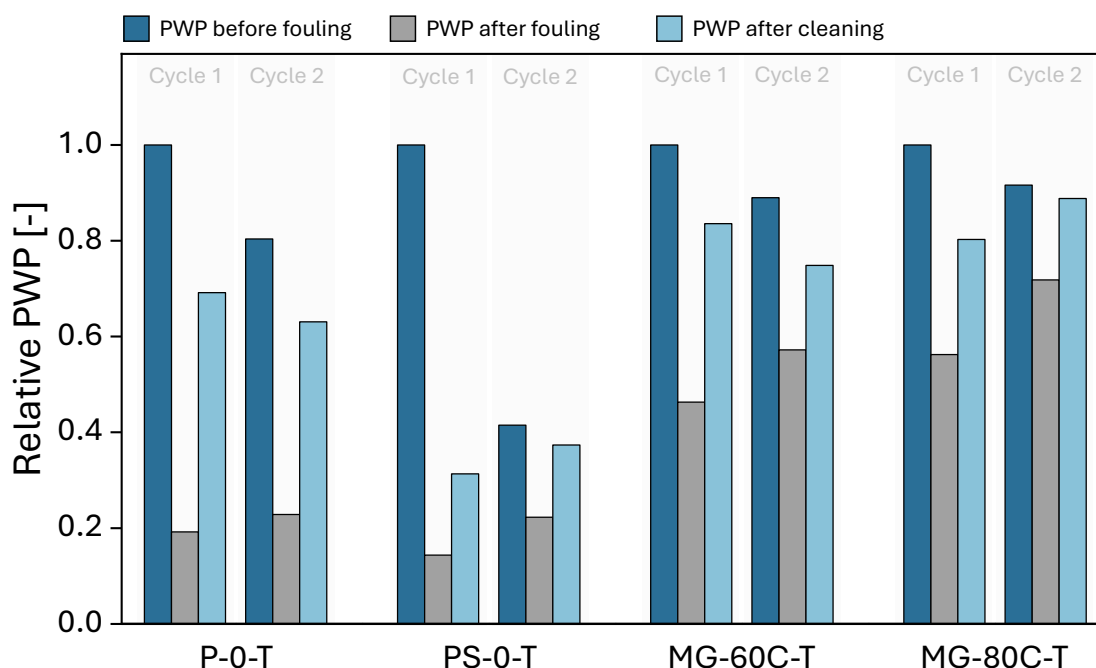


Figure 5.9: Effectiveness of the self-cleaning procedure. Fouling experiments were conducted in cross-flow with at a constant TMP of 1 bar with a gelatin solution (0.5 g L^{-1} in PBS buffer, pH 7.4). PWP measurements were conducted directly before and after gelatin filtration, as well as directly after the cleaning protocol. In between cycles, membranes were stored at 5°C for 6 d.

Table 5.6: PWP of the different membranes before and after enzyme immobilization.

	P-0-T LMH/bar	PS-0-T LMH/bar	MG-60C-T LMH/bar	MG-80C-T LMH/bar
PWP before trypsin	71	95	33	15
PWP after trypsin	70	72	25	10

immobilized trypsin on the surface of the membrane. This finding is unexpected, as PES lacks functional groups to which the cross-linker GA can attach. However, it has been reported that GA can self-polymerize and form so-called poly-GA [Mign2004]. Enzymes and proteins can react with the poly-GA [Mign2004], which could eventually lead to the formation of a trypsin-GA film that adsorbs to the membrane surface independent of the presence of functional groups in PES. Interestingly, the PWP before and

after enzyme immobilization is similar indicating no build up of an enzyme layer, contradicting the self-cleaning effect. However, it must be noted that the PWP of the specific moduled analyzed is lower than the average PWP for P-0 membranes, which is around 100 LMH/bar (see Figure 5.4).

For the unmodified PS-SMA membrane PS-0-T, permeability was reduced to about 20 % of the initial PWP. However, PWP only increased to 30 % after cleaning. No significant changes were observed in the second cycle. In contrast to P-0-T, PS-0-T shows no strong presence of immobilized trypsin. One possibility is that the more negative surface of the membrane (see 5.4) may repel the poly-GA owed to the presence of negatively charged carboxylic acid groups in poly-GA [Marg1980]. Therefore, little GA can adsorb to the surface of the membrane and later react with the amine groups in the trypsin. Nonetheless, PWP measurements before and after enzyme immobilization demonstrate increased membrane resistance, indicating an enzyme layer's deposition. However, the enzyme immobilization may only occur due to electrostatic adsorption since the charge of trypsin is positive at the immobilization pH (IEP trypsin 10.2–10.8). Indeed, it has been demonstrated that covalently immobilized trypsin is more stable than physically adsorbed trypsin due to hydraulic washing and desorption [Shi2011]. Nonetheless, more research should be conducted to confirm this theory.

The microgel-coated membranes MG-60C-T and MG-80C-T both show decreased fouling as indicated by a more moderate decrease in PWP (45 and 55 % of the initial permeability for MG-60C-T and MG-80C-T, respectively). After the self-cleaning protocol, both membranes recovered to approximately 80 % of their initial PWP with a further 10 % increase after six days in storage. After the second fouling cycle, the PWP of MG-60C-T decreases to 55 % and then increases to 75 % after cleaning. MG-80C-T performs better, only reducing its PWP to 70 % after fouling and recovering to approximately 89 % of its initial PWP. While the PWP of the membranes is further reduced after the enzyme immobilization, the results demonstrate the efficacy of the microgel and enzyme coating in not only reducing fouling but also acting as a responsive self-cleaning barrier.

5.4 Conclusion

This study introduces a straightforward method for fabricating low-fouling hollow fiber membranes through the covalent attachment of amine-functionalized p(NIPAM-co-AEMA) microgels to the membrane surface. A membrane support fabricated from a blend of PES and SMA enables the subsequent immobilization of the microgels without the need for harsh chemicals or complex procedures. PES-SMA membranes were coated at 60 and 80 °C (MG-60C and MG-80C, respectively). As a reference, unmodified PES and PES-SMA membranes (P-0 and PS-0, respectively) were evaluated as well. The initial characterization of the modified membranes confirmed successful microgel immobilization. SEM imaging revealed the presence of a uniform microgel layer covering the membrane surface, and zeta potential measurements indicated a positive shift in the zeta potential consistent with the introduction of amine functionalities from the microgels. Microgel-coated membranes also exhibited a decrease in PWP, consistent with the formation of a microgel layer. Extensive fouling experiments demonstrated the enhanced antifouling properties of the microgel-coated membranes. Flux stepping experiments were conducted to determine the J_{th} of the different membranes. Cyclic filtration tests BSA as the model foulant showed that the modified membranes maintained higher permeability over multiple cycles compared to unmodified membranes. The unmodified PS-0 membrane also showed an improved performance compared to the P-0 membrane, which was attributed to an enhanced electrostatic repulsion of the negatively charged BSA. Static adsorption experiments using fluorescently labeled BSA supported these findings, revealing substantially lower protein adsorption on the PES-SMA unmodified and microgel-coated membranes. Further cyclic experiments were conducted using the protein gelatin type A. Under the process conditions used, gelatin is near its IEP, which is known to exacerbate fouling. In this case, the microgel-coated membranes outperformed the unmodified ones, which rapidly lost their permeability and could not complete the filtration cycles.

Moreover, the covalent attachment of the proteolytic enzyme trypsin en-

abled the active removal of adsorbed proteins from the membrane surface. In the case of the microgel-modified membranes, this was achieved through the cross-linker GA, which reacts with amine groups in the microgels and the enzyme. Interestingly, PES unmodified membranes also demonstrated the presence of immobilized trypsin, which was theorized to be due to the self-polymerization of GA, which may lead to the formation of a thin poly-GA layer. Nonetheless, microgel-modified membranes with trypsin recovered up to 90 % of their initial PWP after self-cleaning in two repeated fouling cycles, demonstrating the enzyme immobilisation's efficacy.

Overall, the presented approach offers a practical and scalable solution for enhancing the antifouling properties of hollow fiber membranes. The covalent attachment of functionalized microgels provides a stable modification that can withstand operational conditions without apparent loss of the coating. Future work could optimize coating conditions as to reduce the effect of the microgel and enzyme coating on the membrane resistance. Other options that can be explored is the use of more permeable membranes, from which their separation performance can be tailored by adjusting the coating parameters of the microgels.

6

Conclusion and Future Perspectives

6.1 Conclusion

This thesis has explored innovative techniques for fabricating biocatalytic functional hollow fiber membranes, advancing the field of enzymatic membrane reactors. Enzymes, as biocatalysts, operate under mild conditions (physiological pH and temperature), which can significantly lower energy costs while maintaining product quality. However, the high cost of the enzymes and their susceptibility to degradation under non-optimal conditions are especially challenging, so methods for improving their stability are essential. Enzyme immobilization on the membrane supports is a well-established strategy that improves enzyme stability and integrates enzymatic reactions with product separation and enzyme recovery, leading to a process intensification.

However, common materials used for membrane fabrication do not have the characteristics required for enzyme immobilization. Therefore, this thesis discusses the use of novel materials and modification techniques to overcome the limitations of traditional membrane materials. Additionally, each method discussed also adds beneficial properties to the membrane, such as ion selectivity or reduced fouling propensity.

Chapter 3 presented a method to fabricate free-standing, defect-free, and mechanically stable biocatalytic polyelectrolyte complex (PEC) hol-

low fiber membranes via salt-dilution induced phase separation. By utilizing an all-aqueous phase inversion process, the enzyme alkaline phosphatase (ALP) was directly incorporated into the polymer solution, allowing for in situ entrapment during membrane formation. This approach avoids the use of repro-toxic and environmentally harmful organic solvents, which prevents a direct enzyme integration. To fabricate the membranes, a polymer solution containing poly(sodium-4-styrenesulfonate) (PSS), poly(diallyldimethylammonium chloride) (PDADMAC), KBr, and ALP was extruded through a spinneret with a mixture of water and glycerol as lumen fluid into a water coagulation bath at 0°C. The mechanical stability of the fibers is tuned by subsequent photo-induced cross-linking using 4,4'-diazidostilbene-2,2'-difulfonate tetrahydrate (DAS) and UV light. The resulting membranes had a molecular weight cut-off of 400 Da, a pure water permeance of 1.5 LMH/bar, and a burst pressure of 7 bar. The immobilization of ALP proved successful, as the immobilized showed similar catalytic activity to that of the free enzyme, and both storage and thermal stability were improved. Moreover, the enzymes retained 70 % of their initial activity after UV cross-linking with DAS and maintained their catalytic activity after repeated wash and filtration cycles. This work highlighted the versatility of the fabrication of polyelectrolyte complex hollow fiber membranes via the salt-dilution induced phase separation, serving as a platform technology for the fabrication of porous, selective and bioactive membranes.

Chapter 4 explored three novel single-step methods for modifying membranes with polyelectrolyte coatings as an alternative to the time consuming layer-by-layer technique. This was achieved by the addition of high salt concentrations in the coating solution, which suppressed the complexation of the polyelectrolytes prior to the coating. Then, the controlled reduction of the salt concentration during the coating triggers the formation of a polyelectrolyte complex layer on the membrane. Three coating methods are proposed: (1) In Interfacial Complexation (IC), the polyelectrolyte solution coats the membrane and is subsequently precipitated by flushing with water. (2) Diffusive Desalination (DDS) uses the concentration difference between the coating solution in the lumen and a water stream in the shell side to re-

move salt ions continuously. (3) In Polyelectrolyte Concentration (PC), the polyelectrolyte solution is coated at a constant flux. Here, the membrane retains the polyelectrolyte while ions permeate through. First, the coating methods were evaluated their ability to produce nanofiltration membranes, which varies depending on the coating method used. With PC, membranes with up to 79 % MgCl_2 rejection and a permeability of 30 LMH/bar are obtained. Furthermore, in situ functionalization was demonstrated by adding enzymes to the coating solution. The DDS method successfully immobilized enzymes through adsorption via electrostatic interactions, providing a straightforward approach for achieving membranes with biocatalytic activity.

Chapter 5 introduced a novel and straightforward method for fabricating low-fouling and self-cleaning hollow fiber membranes by immobilizing amine-functionalized microgels onto a blended polyethersulfone (PES) and styrene maleic anhydride (SMA) support. The SMA provided reactive anhydride groups that reacted with amine groups in poly(N-isopropylacrylamide)-based microgels incorporating 2-aminoethyl methacrylate hydrochloride (AEMA), forming stable imide bonds. Membrane characterization confirmed successful microgel immobilization. The hydrophilic microgel layer formed a robust hydration layer on the membrane surface, significantly enhancing resistance to both reversible and irreversible fouling. Antifouling properties were assessed through static protein adsorption tests and cyclic constant-flux filtration using bovine serum albumin (BSA) and gelatin as model foulants. Additionally, the covalent attachment of the proteolytic enzyme trypsin also enabled the active removal of adsorbed proteins from the membrane surface. Although, the immobilization of microgel and enzymes significantly reduced the permeability of the membranes, this work highlighted the versatility of SMA as a functional blending additive in membrane fabrication and demonstrated the effectiveness of microgel and trypsin coatings as antifouling and self-cleaning barrier. Nonetheless, further optimization of the coating conditions must be conducted.

In conclusion, this work presented three different novel techniques which can be used to obtain functional and biocatalytic hollow fiber membranes. Each chapter demonstrates the integration of enzymes and functional ma-

terials into hollow fiber membranes not only enhances enzyme stability but also introduces new functionalities beneficial to separation processes.

6.2 Future perspectives

While this thesis explored different types of enzyme immobilization techniques in membranes and discussed the potential use of biocatalytic membranes for biomedical applications and water treatment, two main challenges remain for their practical use in large-scale applications: the high production costs and the limited stability of the enzymes. Although there are continuous efforts to reduce the costs of enzyme production, membrane researchers should focus on identifying processes which could significantly benefit from biocatalytic membranes but do not rely on the long-term stability of the enzyme.

For instance, applications in the biomedical and pharmaceutical fields offer great opportunities, as membranes are often used as single-use products, which the long-term stability of the enzyme would not limit. Moreover, these processes can greatly benefit from the mild conditions in which the enzymes operate and their specificity to their respective substrate/product. For example, integrating the enzyme carbonic anhydrase (CA) into hollow fiber oxygenator membranes could significantly enhance carbon dioxide removal rates in extracorporeal membrane CO₂ removal (ECCO2R) devices. An improvement in CO₂ removal rate would lead to a reduction in the required membrane area, and as a consequence, blood damage would be reduced, improving the outcome of the patients. Another therapeutic application of biocatalytic membranes is the detoxification of blood, which can be achieved by the immobilization of the enzyme ALP on hemodialysis membranes (see Chapter 3 and Chapter 4).

Nonetheless, industrial applications could also benefit from enzyme–membrane systems. For instance, CA can accelerate CO₂ absorption in carbon capture processes, even at low concentrations. Moreover, in the food and beverage industry, biocatalytic membranes can be used to clarify fruit juice and beer thanks to the immobilization of pectinase and pa-

pain, respectively. Furthermore, biocatalytic membranes in the food and beverage industry can also benefit greatly from antifouling properties and the better hydrodynamic conditions of hollow fibre membranes, which is the fabrication method described in Chapter 5.

Nonetheless, challenges related to scaling up, enzyme stability over extended periods, and production costs must be addressed. Advances in enzyme engineering (such as developing more stable enzyme variants or enhancing immobilization techniques to improve long-term activity) could make industrial applications more feasible. Moreover, achieving biocatalytic membranes with high enzyme loading and activity, high permeability, excellent separation performance, and robust long-term stability is essential for their successful implementation. Addressing these challenges will require interdisciplinary collaborations among materials scientists, biotechnologist, and process engineers to innovate and optimize enzyme integration into membrane systems.

Bibliography

- [Akso2019] C. Aksoy, P. Kaner, A. Asatekin, and P. Z. Çulfaz-Emecen. “Co-deposition of stimuli-responsive microgels with foulants during ultrafiltration as a fouling removal strategy”. *ACS Applied Materials and Interfaces*, vol. 11. 20 Jan. 2019. doi: [10.1021/acsami.9B03217](https://doi.org/10.1021/acsami.9B03217) (cit. on p. 75).
- [Ang2007] W. S. Ang and M. Elimelech. “Protein (bsa) fouling of reverse osmosis membranes: Implications for wastewater reclamation”. *Journal of Membrane Science*, vol. 296. 1-2 Jan. 2007. doi: [10.1016/j.memsci.2007.03.018](https://doi.org/10.1016/j.memsci.2007.03.018) (cit. on p. 96).
- [B2021] A. K. B, S. Lindhoud, and W. M. de Vos. “Hot-pressed polyelectrolyte complexes as novel alkaline stable monovalent-ion selective anion exchange membranes”. *Journal of Colloid and Interface Science*, vol. 593. Jul. 2021. doi: [10.1016/j.jcis.2021.02.077](https://doi.org/10.1016/j.jcis.2021.02.077) (cit. on p. 13).
- [Baig2020] M. I. Baig, E. N. Durmaz, J. D. Willott, W. M. de Vos, M. I. Baig, E. N. Durmaz, J. D. Willott, and W. M. de Vos. “Sustainable membrane production through polyelectrolyte complexation induced aqueous phase separation”. *Advanced Functional Materials*, vol. 30. 5 Jan. 2020. doi: [10.1002/adfm.201907344](https://doi.org/10.1002/adfm.201907344) (cit. on pp. 12, 27, 55).
- [Baig2021] M. I. Baig, P. P. I. Sari, J. Li, J. D. Willott, and W. M. de Vos. “Sustainable aqueous phase separation membranes prepared through mild ph shift induced polyelectrolyte complexation of pss and pei”. *Journal of Membrane Science*, vol. 625. May 2021. doi: [10.1016/j.memsci.2021.119114](https://doi.org/10.1016/j.memsci.2021.119114) (cit. on pp. 13, 27, 55).

- [Baig2022] M. I. Baig, M. Pejman, J. D. Willott, A. Tiraferri, and W. M. D. Vos. "Polyelectrolyte complex hollow fiber membranes prepared via aqueous phase separation". *ACS applied polymer materials*, vol. 4. 2 Feb. 2022. doi: [10.1021/acsapm.1C01464](https://doi.org/10.1021/acsapm.1C01464) (cit. on pp. 28, 41, 55).
- [Bake2023] R. W. Baker. "Membrane technology and applications". *Membrane Technology and Applications*. Jan. 2023. doi: [10.1002/9781119686026](https://doi.org/10.1002/9781119686026) (cit. on pp. 1, 6–11, 14, 15).
- [Barb2022] N. H. Barbhuiya, U. Misra, and S. P. Singh. "Biocatalytic membranes for combating the challenges of membrane fouling and micropollutants in water purification: A review". *Chemosphere*, vol. 286. Pt 2 Jan. 2022. doi: [10.1016/J.CHEMOSPHERE.2021.131757](https://doi.org/10.1016/J.CHEMOSPHERE.2021.131757) (cit. on p. 19).
- [Bart1996] R. A. Bartsch and J. D. Way. "Chemical separations with liquid membranes: An overview". *ACS Symposium Series*, vol. 642. 1996. doi: [10.1021/bk-1996-0642.ch001](https://doi.org/10.1021/bk-1996-0642.ch001) (cit. on p. 8).
- [Bart2018] M. Barth, M. Wiese, W. Ogieglo, D. Go, A. J. C. Kuehne, and M. Wessling. "Monolayer microgel composite membranes with tunable permeability". *Journal of Membrane Science*, vol. 555. Jan. 2018. doi: [10.1016/j.memsci.2018.03.037](https://doi.org/10.1016/j.memsci.2018.03.037) (cit. on p. 75).
- [Bäth2023] S. Bäther, C. S. Hundschell, H. Kieserling, and A. M. Wagemans. "Impact of the solvent properties on molecular interactions and phase behaviour of alginate-gelatin systems". *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 656. Jan. 2023. doi: [10.1016/j.colsurfa.2022.130455](https://doi.org/10.1016/j.colsurfa.2022.130455) (cit. on p. 95).
- [Bell2021] D. J. Bell, S. Ludwanowski, A. Lüken, B. Sarikaya, A. Walther, and M. Wessling. "Hydrogel membranes made from crosslinked microgel multilayers with tunable density". *Journal of Membrane Science*, vol. 620. Jan. 2021. doi: [10.1016/j.memsci.2020.118912](https://doi.org/10.1016/j.memsci.2020.118912) (cit. on p. 75).
- [Bhat2004] A. Bhattacharya and B. N. Misra. "Grafting: A versatile means to modify polymers: Techniques, factors and applications". *Progress in Polymer Science*, vol. 29. 8 Aug. 2004. doi: [10.1016/j.progpolymsci.2004.05.002](https://doi.org/10.1016/j.progpolymsci.2004.05.002) (cit. on p. 17).

- [Blac2014] K. A. Black, D. Priftis, S. L. Perry, J. Yip, W. Y. Byun, and M. Tirrell. "Protein encapsulation via polypeptide complex coacervation". *ACS macro letters*, vol. 3. 10 Oct. 2014. doi: [10.1021/mz500529v](https://doi.org/10.1021/mz500529v) (cit. on p. 26).
- [Bloc2017] W. C. Blocher and S. L. Perry. "Complex coacervate-based materials for biomedicine". *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, vol. 9. 4 Jul. 2017. doi: [10.1002/wnan.1442](https://doi.org/10.1002/wnan.1442) (cit. on p. 27).
- [Boud2010] T. Boudou, T. Crouzier, K. Ren, G. Blin, and C. Picart. "Multiple functionalities of polyelectrolyte multilayer films: New biomedical applications". *Advanced Materials*, vol. 22. 4 Jan. 2010. doi: [10.1002/adma.200901327](https://doi.org/10.1002/adma.200901327) (cit. on p. 26).
- [Bour2016] D. Bourguignon, *Eu policy and legislation on chemicals: Overview, with a focus on reach*, 2016. doi: [10.2861/791277](https://doi.org/10.2861/791277) (cit. on p. 11).
- [Broe1980] L. Broens, F. W. Altena, C. A. Smolders, and D. M. Koenhen. "Asymmetric membrane structures as a result of phase separation phenomena". *Desalination*, vol. 32. C Jan. 1980. doi: [10.1016/S0011-9164\(00\)86004-X](https://doi.org/10.1016/S0011-9164(00)86004-X) (cit. on p. 11).
- [Brug2009] B. V. D. Bruggen. "Chemical modification of polyethersulfone nanofiltration membranes: A review". *Journal of Applied Polymer Science*, vol. 114. 1 Oct. 2009. doi: [10.1002/app.30578](https://doi.org/10.1002/app.30578) (cit. on pp. 16, 17, 75).
- [Cado1980] J. E. Cadotte, R. J. Petersen, R. E. Larson, and E. E. Erickson. "A new thin-film composite seawater reverse osmosis membrane". *Desalination*, vol. 32. C Jan. 1980. doi: [10.1016/S0011-9164\(00\)86003-8](https://doi.org/10.1016/S0011-9164(00)86003-8) (cit. on p. 16).
- [Chen2013] X. Chen, S. Bi, C. Shi, Y. He, L. Zhao, and L. Chen. "Temperature-sensitive membranes prepared from blends of poly(vinylidene fluoride) and poly(n-isopropylacrylamides) microgels". *Colloid and Polymer Science*, vol. 291. 10 Jan. 2013. doi: [10.1007/S00396-013-2985-Y](https://doi.org/10.1007/S00396-013-2985-Y) (cit. on p. 75).
- [Cho2014] E. H. Cho and J. W. Rhim. "Preparation of a new charged nanofiltration membrane based on polyelectrolyte complex by forced fouling induction for a household water purifier". *Macromolecular Re-*

- search* 2015 23:2, vol. 23. 2 Dec. 2014. doi: [10.1007/S13233-015-3017-1](https://doi.org/10.1007/S13233-015-3017-1) (cit. on p. 12).
- [Clec2003] P. L. Clech, B. Jefferson, I. S. Chang, and S. J. Judd. "Critical flux determination by the flux-step method in a submerged membrane bioreactor". *Journal of Membrane Science*, vol. 227. 1-2 Dec. 2003. doi: [10.1016/j.memsci.2003.07.021](https://doi.org/10.1016/j.memsci.2003.07.021) (cit. on p. 89).
- [Così2013] R. D. Cosimo, J. M. Auliffe, A. J. Poulouse, and G. Bohlmann. "Industrial use of immobilized enzymes". *Chemical Society reviews*, vol. 42. 15 Jul. 2013. doi: [10.1039/c3cs35506c](https://doi.org/10.1039/c3cs35506c) (cit. on p. 19).
- [Das1998] N. Das, A. M. Kayastha, and O. P. Malhotra. "Immobilization of urease from pigeonpea (*cajanus cajan* L.) in polyacrylamide gels and calcium alginate beads". *Biotechnology and Applied Biochemistry*, vol. 27. 1 Feb. 1998. doi: [10.1111/j.1470-8744.1998.tb01371.x](https://doi.org/10.1111/j.1470-8744.1998.tb01371.x) (cit. on p. 23).
- [Datt2013] S. Datta, L. R. Christena, and Y. R. S. Rajaram. "Enzyme immobilization: An overview on techniques and support materials". *3 Biotech*, vol. 3. 1 Jun. 2013. doi: [10.1007/s13205-012-0071-7](https://doi.org/10.1007/s13205-012-0071-7) (cit. on pp. 21–23).
- [Dech1991] G. Decher and J.-D. .-. Hong. "Buildup of ultrathin multilayer films by a self-assembly process, 1 consecutive adsorption of anionic and cationic bipolar amphiphiles on charged surfaces". *Makromolekulare Chemie. Macromolecular Symposia*, vol. 46. 1 Jun. 1991. doi: [10.1002/masy.19910460145](https://doi.org/10.1002/masy.19910460145) (cit. on p. 12).
- [Dech1997] G. Decher. "Fuzzy nanoassemblies: Toward layered polymeric multicomposites". *Science*, vol. 277. 5330 1997. doi: [10.1126/science.277.5330.1232](https://doi.org/10.1126/science.277.5330.1232) (cit. on pp. 12, 54).
- [Dech2017] J. Dechnik, J. Gascon, C. J. Doonan, C. Janiak, and C. J. Sumby. "Mixed-matrix membranes". *Angewandte Chemie (International ed. in English)*, vol. 56. 32 Aug. 2017. doi: [10.1002/anie.201701109](https://doi.org/10.1002/anie.201701109) (cit. on p. 17).
- [dGroo2015] J. de Grooth, B. Haakmeester, C. Wever, J. Potreck, W. M. de Vos, and K. Nijmeijer. "Long term physical and chemical stability of poly-electrolyte multilayer membranes". *Journal of Membrane Science*, vol. 489. Jan. 2015. doi: [10.1016/j.memsci.2015.04.031](https://doi.org/10.1016/j.memsci.2015.04.031) (cit. on p. 75).

- [Díez2020] B. Díez and R. Rosal. “A critical review of membrane modification techniques for fouling and biofouling control in pressure-driven membrane processes”. *Nanotechnology for Environmental Engineering* 2020 5:2, vol. 5. 2 Jun. 2020. doi: [10.1007/s41204-020-00077-x](https://doi.org/10.1007/s41204-020-00077-x) (cit. on p. 75).
- [Dizg2018] N. Dizge, R. Epsztein, W. Cheng, C. J. Porter, and M. Elimelech. “Biocatalytic and salt selective multilayer polyelectrolyte nanofiltration membrane”. *Journal of Membrane Science*, vol. 549. 2018. doi: [10.1016/j.memsci.2017.12.026](https://doi.org/10.1016/j.memsci.2017.12.026) (cit. on pp. 16, 54).
- [Drew2010] A. Drews. “Membrane fouling in membrane bioreactors—characterisation, contradictions, cause and cures”. *Journal of Membrane Science*, vol. 363. 1-2 Nov. 2010. doi: [10.1016/j.memsci.2010.06.046](https://doi.org/10.1016/j.memsci.2010.06.046) (cit. on pp. 89, 90).
- [dS Me2024] F. B. de S. Mendes, P. Saha, F. M. F., D. Zafar, J. Rubner, M. Wessling, C. P. Borges, and H. Roth. “Scalable fabrication of amine-functionalized microgel composite membranes and their gas permeation characteristics”. *Journal of Membrane Science*, vol. 692. Jan. 2024. doi: [10.1016/j.memsci.2023.122315](https://doi.org/10.1016/j.memsci.2023.122315) (cit. on p. 80).
- [Durm2020] E. N. Durmaz, M. I. Baig, J. D. Willott, and W. M. D. Vos. “Polyelectrolyte complex membranes via salinity change induced aqueous phase separation”. *ACS Applied Polymer Materials*, vol. 2. 7 Jul. 2020. doi: [10.1021/acsapm.0C00255](https://doi.org/10.1021/acsapm.0C00255) (cit. on pp. 13, 26, 27, 55).
- [Durm2021] E. N. Durmaz, S. Sahin, E. Virga, S. D. Beer, L. C. D. Smet, and W. M. D. Vos. “Polyelectrolytes as building blocks for next-generation membranes with advanced functionalities”. *ACS Applied Polymer Materials*, vol. 3. 9 Sep. 2021. doi: [10.1021/acsapm.1C00654](https://doi.org/10.1021/acsapm.1C00654) (cit. on pp. 27, 54).
- [Dwev2016] A. Dwevedi. *Enzyme immobilization: Advances in industry, agriculture, medicine, and the environment*. Springer International Publishing, Jan. 2016. ISBN: 9783319414188. doi: [10.1007/978-3-319-41418-8](https://doi.org/10.1007/978-3-319-41418-8) (cit. on pp. 20–23).

- [Emon2021] S. Emonds, J. Kamp, J. Borowec, H. Roth, and M. Wessling. "Polyelectrolyte complex tubular membranes via a salt dilution induced phase inversion process". *Advanced Engineering Materials*, vol. 23. 5 2021. DOI: [10.1002/adem.202001401](https://doi.org/10.1002/adem.202001401) (cit. on pp. 28, 41, 42, 45, 55).
- [Emon2022] S. Emonds, J. Kamp, R. Viermann, A. Kalde, H. Roth, and M. Wessling. "Open and dense hollow fiber nanofiltration membranes through a streamlined polyelectrolyte-based spinning process". *Journal of Membrane Science*, vol. 644. Feb. 2022. DOI: [10.1016/j.memsci.2021.120100](https://doi.org/10.1016/j.memsci.2021.120100) (cit. on pp. 54, 63).
- [Fiel1995] R. W. Field, D. Wu, J. A. Howell, and B. B. Gupta. "Critical flux concept for microfiltration fouling". *Journal of Membrane Science*, vol. 100. 3 Apr. 1995. DOI: [10.1016/0376-7388\(94\)00265-z](https://doi.org/10.1016/0376-7388(94)00265-z) (cit. on p. 89).
- [Fiel2011] R. W. Field and G. K. Pearce. "Critical, sustainable and threshold fluxes for membrane filtration with water industry applications". *Advances in Colloid and Interface Science*, vol. 164. 1-2 May 2011. DOI: [10.1016/j.cis2010.12.008](https://doi.org/10.1016/j.cis2010.12.008) (cit. on p. 89).
- [Figo2014] A. Figoli, T. Marino, S. Simone, E. D. Nicolò, X. M. Li, T. He, S. Tornaghi, and E. Drioli. "Towards non-toxic solvents for membrane preparation: A review". *Green Chemistry*, vol. 16. 9 Aug. 2014. DOI: [10.1039/c4gc00613e](https://doi.org/10.1039/c4gc00613e) (cit. on p. 11).
- [Gang2009] S. Ganguli, K. Yoshimoto, S. Tomita, H. Sakuma, T. Matsuoka, K. Shiraki, and Y. Nagasaki. "Regulation of lysozyme activity based on thermotolerant protein/smart polymer complex formation". *Journal of the American Chemical Society*, vol. 131. 18 May 2009. DOI: [10.1021/ja900786z](https://doi.org/10.1021/ja900786z) (cit. on p. 26).
- [Garc2011] C. Garcia-Galan, Á. Berenguer-Murcia, R. Fernandez-Lafuente, and R. C. Rodrigues. "Potential of different enzyme immobilization strategies to improve enzyme performance". *Advanced Synthesis Catalysis*, vol. 353. 16 Nov. 2011. DOI: [10.1002/adsc.201100534](https://doi.org/10.1002/adsc.201100534) (cit. on p. 20).
- [Gin2011] D. L. Gin and R. D. Noble. "Designing the next generation of chemical separation membranes". *Science*, vol. 332. 6030 May 2011. DOI: [10.1126/science.1203771](https://doi.org/10.1126/science.1203771) (cit. on p. 2).

- [Gior2000] L. Giorno and E. Drioli. "Biocatalytic membrane reactors: Applications and perspectives". *Trends in Biotechnology*, vol. 18. 8 Aug. 2000. DOI: [10.1016/S0167-7799\(00\)01472-4](https://doi.org/10.1016/S0167-7799(00)01472-4) (cit. on p. 19).
- [Guil2011] G. R. Guillen, Y. Pan, M. Li, and E. M. Hoek. "Preparation and characterization of membranes formed by nonsolvent induced phase separation: A review". *Industrial and Engineering Chemistry Research*, vol. 50. 7 Apr. 2011. DOI: [10.1021/ir101928r](https://doi.org/10.1021/ir101928r) (cit. on pp. 11, 17).
- [Guis2013] J. M. Guisan. *Immobilization of Enzymes and Cells*. J. M. Guisan, Ed. Humana Press, 2013. ISBN: 978-1-62703-549-1. DOI: [10.1007/978-1-62703-550-7](https://doi.org/10.1007/978-1-62703-550-7) (cit. on pp. 20–22).
- [Guis2020] J. M. Guisan, J. M. Bolivar, F. López-Gallego, and J. Rocha-Martín. *Immobilization of Enzymes and Cells: Methods and Protocols*. J. M. Guisan, J. M. Bolivar, F. López-Gallego, and J. Rocha-Martín, Eds. Springer US, 2020. ISBN: 978-1-0716-0214-0. DOI: [10.1007/978-1-0716-0215-7](https://doi.org/10.1007/978-1-0716-0215-7) (cit. on p. 20).
- [Guo2012] W. Guo, H. H. Ngo, and J. Li. "A mini-review on membrane fouling". *Bioresource Technology*, vol. 122. Jan. 2012. DOI: [10.1016/j.biortech.2012.04.089](https://doi.org/10.1016/j.biortech.2012.04.089) (cit. on p. 75).
- [Hage2023] F. Hagemann, J. Linkhorst, H. Roth, and M. Wessling. "On the balance between albumin loss and removal of middle molecules in dialyzers". *Journal of Membrane Science Letters*, vol. 3. 1 May 2023. DOI: [10.1016/j.memlet.2023.100044](https://doi.org/10.1016/j.memlet.2023.100044) (cit. on p. 47).
- [Helm2024] T. Helmecke, I. I. Rose, M. V. Tsurkan, H. Roth, M. F. Maitz, C. Werner, and M. Wessling. "Poly(styrene-alt-maleic anhydride)-copolymers blended in poly(ether sulfone) membranes as a platform for effective biomolecular surface functionalization". *Journal of Membrane Science*, vol. 689. Jan. 2024. DOI: [10.1016/j.memsci.2023.122050](https://doi.org/10.1016/j.memsci.2023.122050) (cit. on pp. 76, 87, 95).
- [Hołd2015] A. K. Hołda and I. F. Vankelecom. "Understanding and guiding the phase inversion process for synthesis of solvent resistant nanofiltration membranes". *Journal of Applied Polymer Science*, vol. 132. 27 Jul. 2015. DOI: [10.1002/app.42130](https://doi.org/10.1002/app.42130) (cit. on p. 43).

- [Hong2007] S. U. Hong, R. Malaisamy, and M. L. Bruening. "Separation of fluoride from other monovalent anions using multilayer polyelectrolyte nanofiltration membranes". *Langmuir*, vol. 23. 4 Feb. 2007. DOI: [10.1021/la061701y](https://doi.org/10.1021/la061701y) (cit. on p. 54).
- [Horn2019] J. M. Horn, R. A. Kapelner, and A. C. Obermeyer. "Macro- and microphase separated protein-polyelectrolyte complexes: Design parameters and current progress". *Polymers*, vol. 11. 4 Apr. 2019. DOI: [10.3390/polym11040578](https://doi.org/10.3390/polym11040578) (cit. on p. 26).
- [Hu2023] D. Hu, H. Zhao, G. Feng, M. Xu, C. Wang, and Y. Li. "Fabrication of nanofiltration membranes through the deposition of polyethyleneimine on sma-polyethersulfone supports under acid catalysis for cationic dye/salt separation". *Journal of Environmental Chemical Engineering*, vol. 11. 5 Jan. 2023. DOI: [10.1016/j.jece.2023.111055](https://doi.org/10.1016/j.jece.2023.111055) (cit. on p. 76).
- [Huis2000] I. H. Huisman, P. Prádanos, and A. Hernández. "The effect of protein–protein and protein–membrane interactions on membrane fouling in ultrafiltration". *Journal of Membrane Science*, vol. 179. 1-2 Jan. 2000. DOI: [10.1016/S0376-7388\(00\)00501-9](https://doi.org/10.1016/S0376-7388(00)00501-9) (cit. on pp. 75, 96, 97).
- [Ishi2012] T. Ishigami, K. Amano, A. Fujii, Y. Ohmukai, E. Kamio, T. Maruyama, and H. Matsuyama. "Fouling reduction of reverse osmosis membrane by surface modification via layer-by-layer assembly". *Separation and Purification Technology*, vol. 99. Oct. 2012. DOI: [10.1016/j.seppur.2012.08.002](https://doi.org/10.1016/j.seppur.2012.08.002) (cit. on p. 16).
- [Jach2008] B. Jachimska, M. Wasilewska, and Z. Adamczyk. "Characterization of globular protein solutions by dynamic light scattering, electrophoretic mobility, and viscosity measurements". *Langmuir*, vol. 24. 13 Jul. 2008. DOI: [10.1021/la800548p](https://doi.org/10.1021/la800548p) (cit. on p. 95).
- [Jin2003] W. Jin, A. Toutianoush, and B. Tieke. "Use of polyelectrolyte layer-by-layer assemblies as nanofiltration and reverse osmosis membranes". *Langmuir*, vol. 19. 7 Apr. 2003. DOI: [10.1021/la020926f](https://doi.org/10.1021/la020926f) (cit. on p. 12).

- [Jin2019] Z. Jin, L. Du, C. Zhang, Y. Sugiyama, W. Wang, G. Palui, S. Wang, and H. Mattoussi. "Modification of poly(maleic anhydride)-based polymers with h₂n-r nucleophiles: Addition or substitution reaction?", *Bioconjugate Chemistry*, vol. 30. 3 Jan. 2019. DOI: [10.1021/acs.bioconjchem.9B00008](https://doi.org/10.1021/acs.bioconjchem.9B00008) (cit. on p. 76).
- [Jin2020] J. Jin, X. Du, J. Yu, S. Qin, M. He, K. Zhang, and G. Chen. "High performance nanofiltration membrane based on sma-pei cross-linked coating for dye/salt separation". *Journal of Membrane Science*, vol. 611. Jan. 2020. DOI: [10.1016/j.memsci.2020.118307](https://doi.org/10.1016/j.memsci.2020.118307) (cit. on p. 76).
- [Joch2011] P. Jochems, Y. Satyawali, L. Diels, and W. Dejonghe. "Enzyme immobilization on/in polymeric membranes: Status, challenges and perspectives in biocatalytic membrane reactors (bmrs)". *Green Chemistry*, vol. 13. 7 Jul. 2011. DOI: [10.1039/c1gc15178a](https://doi.org/10.1039/c1gc15178a) (cit. on pp. 1, 19, 20, 76).
- [Jonk2023] W. A. Jonkers, E. R. Cornelissen, and W. M. de Vos. "Hollow fiber nanofiltration: From lab-scale research to full-scale applications". *Journal of Membrane Science*, vol. 669. Mar. 2023. DOI: [10.1016/j.memsci.2022.121234](https://doi.org/10.1016/j.memsci.2022.121234) (cit. on p. 16).
- [Jord2022] A. Jordan, C. G. Hall, L. R. Thorp, and H. F. Sneddon. "Replacement of less-preferred dipolar aprotic and ethereal solvents in synthetic organic chemistry with more sustainable alternatives". *Chemical Reviews*, vol. 122. 6 Mar. 2022. DOI: [10.1021/acs.chemrev1C00672](https://doi.org/10.1021/acs.chemrev1C00672) (cit. on p. 11).
- [Kaca1994] V. Kacaniklic, K. Johansson, G. Marko-Varga, L. Gorton, G. Jönsson-Pettersson, and E. Csöregi. "Amperometric biosensors for detection of l- and d-amino acids based on coimmobilized peroxidase and l- and d-amino acid oxidases in carbon paste electrodes". *Electroanalysis*, vol. 6. 5-6 May 1994. DOI: [10.1002/elan.1140060505](https://doi.org/10.1002/elan.1140060505) (cit. on p. 26).
- [Kalt1982] D. G. Kalthod and E. Uckenstein. "Immobilized enzymes: Electrokinetic effects on reaction rates under external diffusion". *Biotechnology and bioengineering*, vol. 24. 10 1982. DOI: [10.1002/bit.260241007](https://doi.org/10.1002/bit.260241007) (cit. on p. 20).

- [Kamp2021a] J. Kamp, S. Emonds, J. Borowec, M. A. R. Toro, and M. Wessling. “On the organic solvent free preparation of ultrafiltration and nanofiltration membranes using polyelectrolyte complexation in an all aqueous phase inversion process”. *Journal of Membrane Science*, vol. 618. Jan. 2021. doi: [10.1016/j.memsci.2020.118632](https://doi.org/10.1016/j.memsci.2020.118632) (cit. on pp. 13, 27, 39, 55, 63, 65).
- [Kamp2021b] J. Kamp, S. Emonds, and M. Wessling. “Designing tubular composite membranes of polyelectrolyte multilayer on ceramic supports with nanofiltration and reverse osmosis transport properties”. *Journal of Membrane Science*, vol. 620. Feb. 2021. doi: [10.1016/j.memsci.2020.118851](https://doi.org/10.1016/j.memsci.2020.118851) (cit. on pp. 54, 65).
- [Kang2007] G. D. Kang, C. J. Gao, W. D. Chen, X. M. Jie, Y. M. Cao, and Q. Yuan. “Study on hypochlorite degradation of aromatic polyamide reverse osmosis membrane”. *Journal of Membrane Science*, vol. 300. 1-2 Aug. 2007. doi: [10.1016/j.memsci.2007.05.025](https://doi.org/10.1016/j.memsci.2007.05.025) (cit. on p. 16).
- [Karg2019] M. Karg, A. Pich, T. Hellweg, T. Hoare, L. A. Lyon, J. J. Crassous, D. Suzuki, R. A. Gumerov, S. Schneider, I. I. Potemkin, and W. Richtering. “Nanogels and microgels: From model colloids to applications, recent developments, and future trends”. *Langmuir*, vol. 35. 19 Jan. 2019. doi: [10.1021/acs.langmuir.8B04304](https://doi.org/10.1021/acs.langmuir.8B04304) (cit. on p. 75).
- [Kayi2013] A. B. Kayitmazer, D. Seeman, B. B. Minsky, P. L. Dubin, and Y. Xu. “Protein–polyelectrolyte interactions”. *Soft Matter*, vol. 9. 9 Feb. 2013. doi: [10.1039/c2sm27002a](https://doi.org/10.1039/c2sm27002a) (cit. on p. 26).
- [Kuma2007] A. Kumar, A. Srivastava, I. Y. Galaev, and B. Mattiasson. “Smart polymers: Physical forms and bioengineering applications”. *Progress in Polymer Science*, vol. 32. 10 Oct. 2007. doi: [10.1016/j.progpolymsci.2007.05.003](https://doi.org/10.1016/j.progpolymsci.2007.05.003) (cit. on p. 26).
- [Labe1993] R. Labeque, C. J. Mullon, J. P. M. Ferreira, R. S. Lees, and R. Langer. “Enzymatic modification of plasma low density lipoproteins in rabbits: A potential treatment for hypercholesterolemia”. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90. 8 Apr. 1993. doi: [10.1073/pnas.90.8.3476](https://doi.org/10.1073/pnas.90.8.3476) (cit. on p. 19).

- [Leva1995] J. M. Levaesalmi and T. J. McCarthy. "Gas permeability of surface-selectively chlorinated poly(4-methyl-1-pentene)". *Macromolecules*, vol. 28. 6 Nov. 1995. doi: [10.1021/ma00110a002](https://doi.org/10.1021/ma00110a002) (cit. on p. 12).
- [Li2018] S. Li, J. Luo, and Y. Wan. "Regenerable biocatalytic nanofiltration membrane for aquatic micropollutants removal". *Journal of Membrane Science*, vol. 549. November 2017 2018. doi: [10.1016/j.memsci.2017.11.075](https://doi.org/10.1016/j.memsci.2017.11.075) (cit. on pp. 16, 54).
- [Li2020] X. Li, Y. Xu, K. Goh, T. H. Chong, and R. Wang. "Layer-by-layer assembly based low pressure biocatalytic nanofiltration membranes for micropollutants removal". *Journal of Membrane Science*, vol. 615. May 2020. doi: [10.1016/j.memsci.2020.118514](https://doi.org/10.1016/j.memsci.2020.118514) (cit. on pp. 16, 47, 54).
- [Li2021] J. Li, G. V. Ewijk, D. J. V. Dijken, J. V. D. Gucht, and W. M. D. Vos. "Single-step application of polyelectrolyte complex films as oxygen barrier coatings". *ACS Applied Materials and Interfaces*, vol. 13. 18 May 2021. doi: [10.1021/acsami.1C05031](https://doi.org/10.1021/acsami.1C05031) (cit. on pp. 13, 55).
- [Li2022] J. Li, A. K. B, G. van Ewijk, D. J. van Dijken, W. M. de Vos, and J. van der Gucht. "A comparison of complexation induced brittleness in pei/pss and pei/napss single-step coatings". *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 648. Sep. 2022. doi: [10.1016/j.colsurfa.2022.129143](https://doi.org/10.1016/j.colsurfa.2022.129143) (cit. on p. 55).
- [Lim2020] K. Lim, M. Sima, R. J. Stewart, and S. D. Minter. "Direct bioelectrocatalysis by redox enzymes immobilized in electrostatically condensed oppositely charged polyelectrolyte electrode coatings". *Analyst*, vol. 145. 4 Feb. 2020. doi: [10.1039/c9an02168j](https://doi.org/10.1039/c9an02168j) (cit. on p. 27).
- [Lin2021] Y. C. Lin, C. M. Chao, D. K. Wang, K. M. Liu, and H. H. Tseng. "Enhancing the antifouling properties of a pvdf membrane for protein separation by grafting branch-like zwitterions via a novel amphiphilic sma-hea linker". *Journal of Membrane Science*, vol. 624. Jan. 2021. doi: [10.1016/j.memsci.2021.119126](https://doi.org/10.1016/j.memsci.2021.119126) (cit. on p. 76).

- [Lung2005] U. Lungwitz, M. Breunig, T. Blunk, and A. Göpferich. "Polyethylenimine-based non-viral gene delivery systems". *European journal of pharmaceuticals and biopharmaceutics : official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik e.V*, vol. 60. 2 Jul. 2005. DOI: [10.1016/j.ejpb.2004.11.011](https://doi.org/10.1016/j.ejpb.2004.11.011) (cit. on p. 26).
- [Luo2018] T. Luo, S. Abdu, and M. Wessling. "Selectivity of ion exchange membranes: A review". *Journal of Membrane Science*, vol. 555. Jun. 2018. DOI: [10.1016/j.memsci.2018.03.051](https://doi.org/10.1016/j.memsci.2018.03.051) (cit. on p. 8).
- [Luo2020] J. Luo, S. Song, H. Zhang, H. Zhang, J. Zhang, and Y. Wan. "Biocatalytic membrane: Go far beyond enzyme immobilization". *Engineering in life sciences*, vol. 20. 11 Nov. 2020. DOI: [10.1002/elsc.202000018](https://doi.org/10.1002/elsc.202000018) (cit. on pp. 19, 20).
- [Ma2016] J. Ma, L. Qin, X. Zhang, and H. Huang. "Temporal evolution of the selectivity-permeability relationship during porous membrane filtration of protein solutions". *Journal of Membrane Science*, vol. 514. Sep. 2016. DOI: [10.1016/j.memsci.2016.05.022](https://doi.org/10.1016/j.memsci.2016.05.022) (cit. on p. 96).
- [Mait2022] S. Maity, B. Mishra, K. Nayak, N. C. Dubey, and B. P. Tripathi. "Zwitterionic microgel based anti(-bio)fouling smart membranes for tunable water filtration and molecular separation". *Materials Today Chemistry*, vol. 24. Jan. 2022. DOI: [10.1016/j.mtchem.2022.100779](https://doi.org/10.1016/j.mtchem.2022.100779) (cit. on p. 75).
- [Mait2023] S. Maity, D. Gaur, B. Mishra, N. C. Dubey, and B. P. Tripathi. "Bactericidal and biocatalytic temperature responsive microgel based self-cleaning membranes for water purification". *Journal of Colloid and Interface Science*, vol. 642. Jan. 2023. DOI: [10.1016/j.jcis.2023.03.095](https://doi.org/10.1016/j.jcis.2023.03.095) (cit. on p. 75).
- [Marg1980] S. Margel and A. Rembaum. "Synthesis and characterization of poly (glutaraldehyde). a potential reagent for protein immobilization and cell separation". *Macromolecules*, vol. 13. 1 1980. DOI: [10.1021/ma60073a004](https://doi.org/10.1021/ma60073a004) (cit. on p. 99).
- [Mart2021] E. W. Martin, T. S. Harmon, J. B. Hopkins, S. Chakravarthy, J. J. Incicco, P. Schuck, A. Soranno, and T. Mittag. "A multi-step nucleation process determines the kinetics of prion-like domain phase

- separation". *Nature Communications* 2021 12:1, vol. 12. 1 Jul. 2021. doi: [10.1038/s41467-021-24727-z](https://doi.org/10.1038/s41467-021-24727-z) (cit. on p. 67).
- [Mazz2021] R. Mazzei, A. Y. Gebreyohannes, E. Papaioannou, S. P. Nunes, I. F. Vankelecom, and L. Giorno. "Enzyme catalysis coupled with artificial membranes towards process intensification in biorefinery-a review". *Bioresource Technology*, vol. 335. Sep. 2021. doi: [10.1016/j.biortech.2021.125248](https://doi.org/10.1016/j.biortech.2021.125248) (cit. on p. 19).
- [McKe1997] S. A. McKelvey, D. T. Clausi, and W. J. Koros. "A guide to establishing hollow fiber macroscopic properties for membrane applications". *Journal of Membrane Science*, vol. 124. 2 Feb. 1997. doi: [10.1016/S0376-7388\(96\)00249-9](https://doi.org/10.1016/S0376-7388(96)00249-9) (cit. on p. 15).
- [Meka2017] V. S. Meka, M. K. Sing, M. R. Pichika, S. R. Nali, V. R. Kolapalli, and P. Kesharwani. "A comprehensive review on polyelectrolyte complexes". *Drug discovery today*, vol. 22. 11 Nov. 2017. doi: [10.1016/j.drudis.2017.06.008](https://doi.org/10.1016/j.drudis.2017.06.008) (cit. on p. 26).
- [Mich1965] A. S. Michaels. "Polyelectrolyte complexes". *Industrial & Engineering Chemistry*, vol. 57, no. 10. 1965. doi: [10.1021/ie50670a007](https://doi.org/10.1021/ie50670a007) (cit. on pp. 12, 26).
- [Mign2004] I. Migneault, C. Dartiguenave, M. J. Bertrand, and K. C. Waldron. "Glutaraldehyde: Behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking". *BioTechniques*, vol. 37. 5 2004. doi: [10.2144/04375rv01](https://doi.org/10.2144/04375rv01) (cit. on p. 98).
- [Mill2014] D. J. Miller, S. Kasemset, L. Wang, D. R. Paul, and B. D. Freeman. "Constant flux crossflow filtration evaluation of surface-modified fouling-resistant membranes". *Journal of Membrane Science*, vol. 452. Jan. 2014. doi: [10.1016/j.memsci.2013.10.037](https://doi.org/10.1016/j.memsci.2013.10.037) (cit. on pp. 89, 90).
- [Mirs1936] A. E. Mirsky and L. Pauling. "On the structure of native, denatured, and coagulated proteins". *Proceedings of the National Academy of Sciences*, vol. 22. 7 Jul. 1936. doi: [10.1073/pnas.22.7.439](https://doi.org/10.1073/pnas.22.7.439) (cit. on p. 48).
- [Mish2022] B. Mishra, S. Biswal, N. C. Dubey, and B. P. Tripathi. "Anti(-bio)fouling nanostructured membranes based on the cross-linked assembly of stimuli-responsive zwitterionic microgels". *ACS Ap-*

- plied Polymer Materials*, vol. 4. 7 Jan. 2022. DOI: [10.1021/acsapm.2C00302](https://doi.org/10.1021/acsapm.2C00302) (cit. on p. 75).
- [Miya1990] T. Miyano, T. Matsuura, D. J. Carlsson, and S. Sourirajan. "Retention of polyvinylpyrrolidone swelling agent in the poly(ether p-phenylenesulfone) ultrafiltration membrane". *Journal of Applied Polymer Science*, vol. 41. 1-2 Jan. 1990. DOI: [10.1002/app.1990.070410132](https://doi.org/10.1002/app.1990.070410132) (cit. on pp. 18, 75).
- [Miza2023] M. M. H. Mizan, M. Rastgar, S. A. Aktij, A. Asad, P. Karami, A. Rahimpour, and M. Sadrzadeh. "Organic solvent-free polyelectrolyte complex membrane preparation: Effect of monomer mixing ratio and casting solution temperature". *Journal of Membrane Science*, vol. 668. Feb. 2023. DOI: [10.1016/j.memsci.2022.121197](https://doi.org/10.1016/j.memsci.2022.121197) (cit. on p. 27).
- [Moha2012] A. W. Mohammad, C. Y. Ng, Y. P. Lim, and G. H. Ng. "Ultrafiltration in food processing industry: Review on application, membrane fouling, and fouling control". *Food and Bioprocess Technology*, vol. 5. 4 May 2012. DOI: [10.1007/S11947-012-0806-9](https://doi.org/10.1007/S11947-012-0806-9) (cit. on p. 16).
- [Moha2015] N. R. Mohamad, N. H. C. Marzuki, N. A. Buang, F. Huyop, and R. A. Wahab. "An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes". *Biotechnology Biotechnological Equipment*, vol. 29. 2 2015. DOI: [10.1080/13102818.2015.1008192](https://doi.org/10.1080/13102818.2015.1008192) (cit. on pp. 21, 22).
- [Moha2021] M. Mohammadifakhr, J. de Grooth, K. Trzaskus, H. D. Roesink, and A. J. Kemperman. "Single-step synthesis of a polyelectrolyte complex hollow-fiber membrane for forward osmosis". *Separation and Purification Technology*, vol. 264. Jun. 2021. DOI: [10.1016/j.seppur.2021.118430](https://doi.org/10.1016/j.seppur.2021.118430) (cit. on p. 54).
- [Moli2021] C. Molina-Fernández and P. Luis. "Immobilization of carbonic anhydrase for co2 capture and its industrial implementation: A review". *Journal of CO2 Utilization*, vol. 47. May 2021. DOI: [10.1016/J.JCOU.2021.101475](https://doi.org/10.1016/J.JCOU.2021.101475) (cit. on p. 19).
- [Mort1991] D. A. Mortimer. "Synthetic polyelectrolytes—a review". *Polymer International*, vol. 25. 1 Jan. 1991. DOI: [10.1002/pi.4990250107](https://doi.org/10.1002/pi.4990250107) (cit. on p. 26).

- [Nels1916] J. M. Nelson and E. G. Griffin. "Adsorption of invertase." *Journal of the American Chemical Society*, vol. 38. 5 May 1916. DOI: [10.1021/ja02262a018](https://doi.org/10.1021/ja02262a018) (cit. on p. 20).
- [Ng2013] L. Y. Ng, A. W. Mohammad, C. P. Leo, and N. Hilal. "Polymeric membranes incorporated with metal/metal oxide nanoparticles: A comprehensive review". *Desalination*, vol. 308. Jan. 2013. DOI: [10.1016/j.desal.2010.11.033](https://doi.org/10.1016/j.desal.2010.11.033) (cit. on p. 17).
- [Nune2020] S. P. Nunes, P. Z. Culfaz-Emecen, G. Z. Ramon, T. Visser, G. H. Koops, W. Jin, and M. Ulbricht. "Thinking the future of membranes: Perspectives for advanced and new membrane materials and manufacturing processes". *Journal of Membrane Science*, vol. 598. Mar. 2020. DOI: [10.1016/j.memsci.2019.117761](https://doi.org/10.1016/j.memsci.2019.117761) (cit. on pp. 2, 9).
- [Oort2012] D. A. Oortgiesen, A. S. Plachokova, C. Geenen, G. J. Meijer, X. F. Walboomers, J. J. V. D. Beucken, and J. A. Jansen. "Alkaline phosphatase immobilization onto bio-gide® and bio-oss® for periodontal and bone regeneration". *Journal of Clinical Periodontology*, vol. 39. 6 Jun. 2012. DOI: [10.1111/j.1600-051x.2012.01877.x](https://doi.org/10.1111/j.1600-051x.2012.01877.x) (cit. on p. 28).
- [Osat2009] T. Osathanon, C. M. Giachelli, and M. J. Somerman. "Immobilization of alkaline phosphatase on microporous nanofibrous fibrin scaffolds for bone tissue engineering". *Biomaterials*, vol. 30. 27 Sep. 2009. DOI: [10.1016/J.BIOMATERIALS.2009.05.022](https://doi.org/10.1016/J.BIOMATERIALS.2009.05.022) (cit. on p. 28).
- [Otte2005] L. G. Otten and W. J. Quax. "Directed evolution: Selecting today's biocatalysts". *Biomolecular Engineering*, vol. 22. 1-3 Jun. 2005. DOI: [10.1016/j.bioeng.2005.02.002](https://doi.org/10.1016/j.bioeng.2005.02.002) (cit. on p. 19).
- [Pein2010] K. V. Peinemann and S. P. Nunes. *Membranes for water treatment*. Wiley-VCH ; [John Wiley, distributor], 2010. ISBN: 978-3-527-63141-4. (Cit. on p. 75).
- [Pekg2022] E. Pekgenc, B. Y. Gul, V. Vatanpour, and I. Koyuncu. "Biocatalytic membranes in anti-fouling and emerging pollutant degradation applications: Current state and perspectives". *Separation and Purification Technology*, vol. 282. Feb. 2022. DOI: [10.1016/j.seppur.2021.120098](https://doi.org/10.1016/j.seppur.2021.120098) (cit. on p. 19).

- [Peng2012] N. Peng, N. Widjojo, P. Sukitpaneemit, M. M. Teoh, G. G. Lipscomb, T. S. Chung, and J. Y. Lai. "Evolution of polymeric hollow fibers as sustainable technologies: Past, present, and future". *Progress in Polymer Science*, vol. 37. 10 Oct. 2012. doi: [10.1016/j.progpolymsci.2012.01.001](https://doi.org/10.1016/j.progpolymsci.2012.01.001) (cit. on pp. 14, 15).
- [Pete2014] E. Peters, R. Masereeuw, and P. Pickkers. "The potential of alkaline phosphatase as a treatment for sepsis-associated acute kidney injury". *Nephron. Clinical practice*, vol. 127. 1-4 Apr. 2014. doi: [10.1159/000363256](https://doi.org/10.1159/000363256) (cit. on pp. 19, 28, 47).
- [Pomp2005] T. Pompe, L. Renner, M. Grimmer, N. Herold, and C. Werner. "Functional films of maleic anhydride copolymers under physiological conditions". *Macromolecular Bioscience*, vol. 5. 9 Jan. 2005. doi: [10.1002/mabi.200500097](https://doi.org/10.1002/mabi.200500097) (cit. on p. 92).
- [Quio1964] F. A. Quiocho and F. M. Richards. "Intermolecular cross linking of a protein in the crystalline state: Carboxypeptidase-a". *Proceedings of the National Academy of Sciences of the United States of America*, vol. 52. 3 1964. doi: [10.1073/pnas.52.3.833](https://doi.org/10.1073/pnas.52.3.833) (cit. on p. 21).
- [Rana2010] D. Rana and T. Matsuura. "Surface modifications for antifouling membranes". *Chemical Reviews*, vol. 110. 4 Jan. 2010. doi: [10.1021/cr800208y](https://doi.org/10.1021/cr800208y) (cit. on pp. 17, 75, 76).
- [Rest2024] M. A. Restrepo, S. Emonds, A. Zhao, F. Karakas, J. Kamp, H. Roth, and M. Wessling. "Self-supporting biocatalytic polyelectrolyte complex hollow fiber membranes via salt-dilution induced phase separation". *Journal of Membrane Science*, vol. 689. Jan. 2024. doi: [10.1016/j.memsci.2023.122157](https://doi.org/10.1016/j.memsci.2023.122157) (cit. on pp. 2, 13).
- [Rome2019] M. Romero-Fernández and F. Paradisi. "General overview on immobilization techniques of enzymes for biocatalysis". *Catalyst Immobilization: Methods and Applications*. Dec. 2019. doi: [10.1002/9783527817290.ch12](https://doi.org/10.1002/9783527817290.ch12) (cit. on p. 23).
- [Sadm2019] K. Sadman, D. E. Delgado, Y. Won, Q. Wang, K. A. Gray, and K. R. Shull. "Versatile and high-throughput polyelectrolyte complex membranes via phase inversion". *ACS applied materials interfaces*, vol. 11. 17 May 2019. doi: [10.1021/acsami.9B02115](https://doi.org/10.1021/acsami.9B02115) (cit. on pp. 27, 55).

- [Sakr2013] O. S. Sakr and G. Borchard. "Encapsulation of enzymes in layer-by-layer (lbl) structures: Latest advances and applications". *Biomacromolecules*, vol. 14. 7 Jul. 2013. doi: [10.1021/bm400198p](https://doi.org/10.1021/bm400198p) (cit. on p. 27).
- [Sarm2017] R. Sarma, M. S. Islam, A. F. Miller, and D. Bhattacharyya. "Layer-by-layer-assembled laccase enzyme on stimuli-responsive membranes for chloro-organics degradation". *ACS Applied Materials and Interfaces*, vol. 9. 17 May 2017. doi: [10.1021/acsami.7B01999](https://doi.org/10.1021/acsami.7B01999) (cit. on p. 54).
- [Scha2015] P. Schaaf and J. B. Schlenoff. "Saloplastics: Processing compact polyelectrolyte complexes". *Advanced Materials*, vol. 27. 15 Apr. 2015. doi: [10.1002/adma.201500176](https://doi.org/10.1002/adma.201500176) (cit. on p. 12).
- [Schm2011] C. Schmitt and S. L. Turgeon. "Protein/polysaccharide complexes and coacervates in food systems". *Advances in Colloid and Interface Science*, vol. 167. 1-2 Sep. 2011. doi: [10.1016/j.cis.2010.10.001](https://doi.org/10.1016/j.cis.2010.10.001) (cit. on p. 26).
- [Schm2018] M. Schmidt, D. Breite, I. Thomas, M. Went, A. Prager, and A. Schulze. "Polymer membranes for active degradation of complex fouling mixtures". *Journal of Membrane Science*, vol. 563. Oct. 2018. doi: [10.1016/j.memsci.2018.06.013](https://doi.org/10.1016/j.memsci.2018.06.013) (cit. on p. 76).
- [Schu2015] A. Schulze, A. Stoelzer, K. Striegler, S. Starke, and A. Prager. "Biocatalytic self-cleaning polymer membranes". *Polymers 2015, Vol. 7, Pages 1837-1849*, vol. 7. 9 Sep. 2015. doi: [10.3390/polym7091485](https://doi.org/10.3390/polym7091485) (cit. on p. 76).
- [Schu2017] A. Schulze, D. Breite, Y. Kim, M. Schmidt, I. Thomas, M. Went, K. Fischer, and A. Prager. "Bio-inspired polymer membrane surface cleaning". *Polymers 2017, Vol. 9, Page 97*, vol. 9. 3 Mar. 2017. doi: [10.3390/polym9030097](https://doi.org/10.3390/polym9030097) (cit. on p. 76).
- [Sen2007] S. Sen, V. V. Dasu, and B. Mandal. "Developments in directed evolution for improving enzyme functions". *Applied biochemistry and biotechnology*, vol. 143. 3 Dec. 2007. doi: [10.1007/s12010-007-8003-4](https://doi.org/10.1007/s12010-007-8003-4) (cit. on p. 20).

- [Sham2012] R. F. Shamoun, H. H. Hariri, R. A. Ghostine, and J. B. Schlenoff. "Thermal transformations in extruded saloplastic polyelectrolyte complexes". *Macromolecules*, vol. 45. 24 Dec. 2012. doi: [10.1021/ma302075p](https://doi.org/10.1021/ma302075p) (cit. on p. 44).
- [She2009] Q. She, C. Y. Tang, Y. N. Wang, and Z. Zhang. "The role of hydrodynamic conditions and solution chemistry on protein fouling during ultrafiltration". *Desalination*, vol. 249. 3 Dec. 2009. doi: [10.1016/j.desal.2009.05.015](https://doi.org/10.1016/j.desal.2009.05.015) (cit. on p. 96).
- [Shel2007] R. A. Sheldon. "Cross-linked enzyme aggregates (clea@s): Stable and recyclable biocatalysts". *Biochemical Society Transactions*, vol. 35. 6 Dec. 2007. doi: [10.1042/bst0351583](https://doi.org/10.1042/bst0351583) (cit. on p. 21).
- [Shel2021] R. A. Sheldon and D. Brady. "Streamlining design, engineering, and applications of enzymes for sustainable biocatalysis". *ACS Sustainable Chemistry and Engineering*, vol. 9. 24 Jun. 2021. doi: [10.1021/acssuschemeng.1C01742](https://doi.org/10.1021/acssuschemeng.1C01742) (cit. on p. 19).
- [Shi2011] Q. Shi, Y. Su, X. Ning, W. Chen, J. Peng, and Z. Jiang. "Trypsin-enabled construction of anti-fouling and self-cleaning polyethersulfone membrane". *Bioresource Technology*, vol. 102. 2 Jan. 2011. doi: [10.1016/j.biortech.2010.08.030](https://doi.org/10.1016/j.biortech.2010.08.030) (cit. on p. 99).
- [Shi2014] X. Shi, G. Tal, N. P. Hankins, and V. Gitis. "Fouling and cleaning of ultrafiltration membranes: A review". *Journal of Water Process Engineering*, vol. 1. Jan. 2014. doi: [10.1016/j.jwpe.2014.04.003](https://doi.org/10.1016/j.jwpe.2014.04.003) (cit. on p. 75).
- [Sigu2018] S. B. Sigurdardóttir, J. Lehmann, S. Ovtar, J. C. Grivel, M. D. Negra, A. Kaiser, and M. Pinelo. "Enzyme immobilization on inorganic surfaces for membrane reactor applications: Mass transfer challenges, enzyme leakage and reuse of materials". *Advanced Synthesis Catalysis*, vol. 360. 14 Jul. 2018. doi: [10.1002/adsc.201800307](https://doi.org/10.1002/adsc.201800307) (cit. on p. 27).
- [Smul2011] V. Smuleac, R. Varma, B. Baruwati, S. Sikdar, and D. Bhattacharyya. "Nanostructured membranes for enzyme catalysis and green synthesis of nanoparticles". *ChemSusChem*, vol. 4. 12 Dec. 2011. doi: [10.1002/cssc.201100211](https://doi.org/10.1002/cssc.201100211) (cit. on p. 54).

- [Star2013] S. Starke, M. Went, A. Prager, and A. Schulze. "A novel electron beam-based method for the immobilization of trypsin on poly(ethersulfone) and poly(vinylidene fluoride) membranes". *Reactive and Functional Polymers*, vol. 73. 5 May 2013. DOI: [10.1016/j.reactfunctpolym.2013.02.013](https://doi.org/10.1016/j.reactfunctpolym.2013.02.013) (cit. on p. 22).
- [Stra1975] H. Strathmann, K. Kock, P. Amar, and R. W. Baker. "The formation mechanism of asymmetric membranes". *Desalination*, vol. 16. 2 Apr. 1975. DOI: [10.1016/S0011-9164\(00\)82092-5](https://doi.org/10.1016/S0011-9164(00)82092-5) (cit. on p. 43).
- [Swar2022] J. Swartjes, M. Storr, M. Rempfer, I. Litzinger, S. Votteler, and K. Philipp, *Blood treatment device comprising alkaline phosphatase*, US Patent App. 17/433,761, Apr. 2022 (cit. on p. 28).
- [Tang2022] K. H. D. Tang, S. S. M. Lock, P. S. Yap, K. W. Cheah, Y. H. Chan, C. L. Yiin, A. Z. E. Ku, A. C. M. Loy, B. L. F. Chin, and Y. H. Chai. "Immobilized enzyme/microorganism complexes for degradation of microplastics: A review of recent advances, feasibility and future prospects". *The Science of the total environment*, vol. 832. Aug. 2022. DOI: [10.1016/j.scitotenv.2022.154868](https://doi.org/10.1016/j.scitotenv.2022.154868) (cit. on p. 26).
- [Thie2018] M. J. Thiele, M. D. Davari, M. König, I. Hofmann, N. O. Junker, T. M. Garakani, L. Vojcic, J. Fitter, and U. Schwaneberg. "Enzyme-polyelectrolyte complexes boost the catalytic performance of enzymes". *ACS Catalysis*, vol. 8. 11 Nov. 2018. DOI: [10.1021/acscatal.8B02935](https://doi.org/10.1021/acscatal.8B02935) (cit. on p. 26).
- [Tira2013] P. Tirado, A. Reisch, E. Roger, F. Boulmedais, L. Jierry, P. Lavalley, J. C. Voegel, P. Schaaf, J. B. Schlenoff, and B. Frisch. "Catalytic saloplastics: Alkaline phosphatase immobilized and stabilized in compacted polyelectrolyte complexes". *Advanced Functional Materials*, vol. 23. 38 Oct. 2013. DOI: [10.1002/adfm.201300117](https://doi.org/10.1002/adfm.201300117) (cit. on pp. 26, 39).
- [Urag2013] T. Uragami, S. Chakraborty, V. Piemonte, and L. D. Paola. "Biocatalytic membrane reactors: Principles, preparation and biotechnological, pharmaceutical and medical applications". *Handbook of Membrane Reactors*, vol. 2. Jan. 2013. DOI: [10.1533/9780857097347.4.846](https://doi.org/10.1533/9780857097347.4.846) (cit. on p. 19).

- [Vana2018] A. Vanangamudi, D. Saeki, L. F. Dumée, M. Duke, T. Vasiljevic, H. Matsuyama, and X. Yang. "Surface-engineered biocatalytic composite membranes for reduced protein fouling and self-cleaning". *ACS Applied Materials and Interfaces*, vol. 10. 32 Aug. 2018. DOI: [10.1021/acsami.8B07945](https://doi.org/10.1021/acsami.8B07945) (cit. on p. 76).
- [vdHors1995] H. C. van der Horst, J. M. Timmer, T. Robbertsen, and J. Leenders. "Use of nanofiltration for concentration and demineralization in the dairy industry: Model for mass transport". *Journal of Membrane Science*, vol. 104. 3 Aug. 1995. DOI: [10.1016/0376-7388\(95\)00041-a](https://doi.org/10.1016/0376-7388(95)00041-a) (cit. on p. 8).
- [vdMare2009] P. van der Marel, A. Zwijnenburg, A. Kemperman, M. Wessling, H. Temmink, and W. van der Meer. "An improved flux-step method to determine the critical flux and the critical flux for irreversibility in a membrane bioreactor". *Journal of Membrane Science*, vol. 332. 1-2 Apr. 2009. DOI: [10.1016/j.memsci.2009.01.046](https://doi.org/10.1016/j.memsci.2009.01.046) (cit. on p. 89).
- [Vinh2013] H. Vinh-Thang and S. Kaliaguine. "Predictive models for mixed-matrix membrane performance: A review". *Chemical Reviews*, vol. 113. 7 Jul. 2013. DOI: [10.1021/cr3003888](https://doi.org/10.1021/cr3003888) (cit. on p. 8).
- [Vito2017] G. Vitola, D. Büning, J. Schumacher, R. Mazzei, L. Giorno, and M. Ulbricht. "Development of a novel immobilization method by using microgels to keep enzyme in hydrated microenvironment in porous hydrophobic membranes". *Macromolecular Bioscience*, vol. 17. 5 Jan. 2017. DOI: [10.1002/mabi.201600381](https://doi.org/10.1002/mabi.201600381) (cit. on p. 76).
- [vLent2022] J. J. van Lente, M. I. Baig, W. M. de Vos, and S. Lindhoud. "Biocatalytic membranes through aqueous phase separation". *Journal of colloid and interface science*, vol. 616. Jun. 2022. DOI: [10.1016/j.jcis.2022.02.094](https://doi.org/10.1016/j.jcis.2022.02.094) (cit. on p. 28).
- [Voet2009] I. K. Voets, A. de Keizer, and M. A. C. Stuart. "Complex coacervate core micelles". *Advances in Colloid and Interface Science*, vol. 147-148. C Mar. 2009. DOI: [10.1016/j.jcis.2008.09.012](https://doi.org/10.1016/j.jcis.2008.09.012) (cit. on p. 12).
- [Wang2013] D. M. Wang and J. Y. Lai. "Recent advances in preparation and morphology control of polymeric membranes formed by nonsolvent induced phase separation". *Current Opinion in Chemical Engineer-*

- ing, vol. 2. 2 May 2013. doi: [10.1016/j.coche.2013.04.003](https://doi.org/10.1016/j.coche.2013.04.003) (cit. on p. 18).
- [Wang2014] Q. Wang and J. B. Schlenoff. "The polyelectrolyte complex/coacervate continuum". *Macromolecules*, vol. 47. 9 May 2014. doi: [10.1021/ma500500q](https://doi.org/10.1021/ma500500q) (cit. on pp. 12, 13, 26, 63).
- [Wang2015] Q. Wang and J. B. Schlenoff. "Single- and multicompartment hollow polyelectrolyte complex microcapsules by one-step spraying". *Advanced Materials*, vol. 27. 12 Mar. 2015. doi: [10.1002/adma.201405376](https://doi.org/10.1002/adma.201405376) (cit. on pp. 13, 63).
- [Wien1996] I. M. Wienk, R. M. Boom, M. A. Beerlage, A. M. Bulte, C. A. Smolders, and H. Strathmann. "Recent advances in the formation of phase inversion membranes made from amorphous or semicrystalline polymers". *Journal of Membrane Science*, vol. 113. 2 May 1996. doi: [10.1016/0376-7388\(95\)00256-1](https://doi.org/10.1016/0376-7388(95)00256-1) (cit. on p. 10).
- [Wies2019] M. Wiese, T. Lohaus, J. Haussmann, and M. Wessling. "Charged microgels adsorbed on porous membranes - a study of their mobility and molecular retention". *Journal of Membrane Science*, vol. 588. Jan. 2019. doi: [10.1016/j.memsci.2019.117190](https://doi.org/10.1016/j.memsci.2019.117190) (cit. on p. 75).
- [Xian2012] T. Xiang, M. Tang, Y. Liu, H. Li, L. L. Li, W. Cao, S. Sun, and C. Zhao. "Preparation and characterization of modified polyethersulfone hollow fiber membranes by blending poly (styrene-alt-maleic anhydride)". *Desalination*, vol. 295. Jun. 2012. doi: [10.1016/j.desal.2012.03.021](https://doi.org/10.1016/j.desal.2012.03.021) (cit. on p. 76).
- [Yan2020] Z. Yan, Y. Zhang, H. Yang, G. Fan, A. Ding, H. Liang, G. Li, N. Ren, and B. V. der Bruggen. "Mussel-inspired polydopamine modification of polymeric membranes for the application of water and wastewater treatment: A review". *Chemical Engineering Research and Design*, vol. 157. Jan. 2020. doi: [10.1016/j.cherd.2020.03.011](https://doi.org/10.1016/j.cherd.2020.03.011) (cit. on p. 75).
- [Yi2024] Z. Yi, L. Zhu, R. Xiong, C. Fang, B. Zhu, L. Zhu, and H. Zeng. "Advanced functional membranes based on amphiphilic copolymers". *Progress in Polymer Science*, vol. 159. Dec. 2024. doi: [10.1016/j.progpolymsci.2024.101907](https://doi.org/10.1016/j.progpolymsci.2024.101907) (cit. on p. 18).

- [Yong2015] J. K. Yong, J. Cui, K. L. Cho, G. W. Stevens, F. Caruso, and S. E. Kentish. "Surface engineering of polypropylene membranes with carbonic anhydrase-loaded mesoporous silica nanoparticles for improved carbon dioxide hydration". *Langmuir : the ACS journal of surfaces and colloids*, vol. 31. 22 Jun. 2015. DOI: [10.1021/acs.langmuir.5B01020](https://doi.org/10.1021/acs.langmuir.5B01020) (cit. on p. 54).
- [Zhan2012] X. Zhang, C. Jiang, M. Cheng, Y. Zhou, X. Zhu, J. Nie, Y. Zhang, Q. An, and F. Shi. "Facile method for the fabrication of robust poly-electrolyte multilayers by post-photo-cross-linking of azido groups". *Langmuir*, vol. 28. 18 May 2012. DOI: [10.1021/la300611g](https://doi.org/10.1021/la300611g) (cit. on p. 43).
- [Zhan2016] R. Zhang, Y. Liu, M. He, Y. Su, X. Zhao, M. Elimelech, and Z. Jiang. "Antifouling membranes for sustainable water purification: Strategies and mechanisms". *Chemical Society Reviews*, vol. 45. 21 Jan. 2016. DOI: [10.1039/c5cs00579e](https://doi.org/10.1039/c5cs00579e) (cit. on pp. 75, 76).
- [Zhan2021] H. Zhang, J. Luo, J. M. Woodley, and Y. Wan. "Confining the motion of enzymes in nanofiltration membrane for efficient and stable removal of micropollutants". *Chemical Engineering Journal*, vol. 421. Oct. 2021. DOI: [10.1016/j.cej.2020.127870](https://doi.org/10.1016/j.cej.2020.127870) (cit. on p. 47).
- [Zhu2007] L. P. Zhu, X. X. Zhang, L. Xu, C. H. Du, B. K. Zhu, and Y. Y. Xu. "Improved protein-adsorption resistance of polyethersulfone membranes via surface segregation of ultrahigh molecular weight poly(styrene-alt-maleic anhydride)". *Colloids and Surfaces B: Biointerfaces*, vol. 57. 2 Jun. 2007. DOI: [10.1016/j.colsurfb.2007.01.021](https://doi.org/10.1016/j.colsurfb.2007.01.021) (cit. on p. 76).
- [Zou2021] D. Zou, S. P. Nunes, I. F. Vankelecom, A. Figoli, and Y. M. Lee. "Recent advances in polymer membranes employing non-toxic solvents and materials". *Green Chemistry*, vol. 23. 24 Dec. 2021. DOI: [10.1039/d1gc03318B](https://doi.org/10.1039/d1gc03318B) (cit. on p. 11).

