THE DEVELOPMENT OF THIN FILMS FOR EFFICIENT CARBON CAPTURE AND STORAGE

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Abstract

The chemical absorption of carbon dioxide (CO₂) into a monoethanolamine (MEA) solvent is the accepted commercial process for industrial CO₂ capture operations. Gas-liquid absorption processes are conducted in absorption towers or membrane contactors, with absorption columns being the preferred industrial method. However, the subsequent desorption of CO₂ from the typical MEA solvent is energy intensive. Alternative solvents, such as potassium carbonate (K₂CO₃), can be more energy efficient and relatively inert in comparison to the alkanolamines, but their slow reaction kinetics limits their application in absorption processes.

The use of a carbonic anhydrase (CA) enzyme as a reaction promoter may overcome the reduced overall absorption rates exhibited by K₂CO₃ relative to MEA. However, these enzymes tend to denature at higher temperatures and would not be suited for use in circulation within a traditional absorber-stripper process. The immobilization of the enzyme within the gas absorber or onto a membrane contactor can increase enzyme stability and avoid thermal denaturation in the stripper. However, immobilization is only effective if the mass transfer of CO₂ through the liquid phase to reach the immobilization substrate does not become rate controlling. The enzyme-aided mass transfer of CO₂ is at its most effective when the enzyme is immobilized at the gas-liquid interface. The immobilization of CA onto a membrane contactor brings it closer to the gas-liquid interface and has yielded more substantial increases in the mass transfer kinetics.

Multiple methods for immobilizing CA onto the surface of a membrane contactor have been proposed, though the layer-by-layer (LbL) technique for enzyme immobilization has not been investigated for enhancing CO₂ absorption operations thus far. LbL assembly has been used effectively on a wide range of polyelectrolytes and proteins for the
fabrication of uniform thin films on different planar surfaces. It is possible to immobilize CA in thin films on a polymeric membrane surface for enhancing CO₂ mass transfer rates to ensure that the CA is at the gas-liquid interface. Enzyme immobilization stabilizes the enzyme structure and preserves its activity over a long period of time, and multiple layers of enzyme can be added to increase the enzyme loading on the membrane surface. However, the coating of polyelectrolytes onto a surface renders the surface more hydrophilic and closes up the pores of the membrane surface, which may cause wetting to occur more easily or the mass transfer to be more hindered by the polyelectrolyte layers.

It was found in this investigation that the deposition of CA as a thin film via LbL assembly on the surface of a flat sheet membrane was able to increase the membrane resistance to wetting by closing up the membrane pores significantly, even though the hydrophilicity of the membrane was increased. The layering of mesoporous silica nanoparticles onto the membrane surface further helped to close up the pores and increase the total enzyme loading onto the flat sheet membrane as compared to a regular multilayer enzyme film. However, the specific enzyme activity was found to be significantly reduced upon the immobilization to the nanoparticles. Hence, it was decided that the LbL film assembly technique would only make use of regular multilayer films when scaling up immobilization onto hollow fiber membranes.

The scaling up of the LbL technique to hollow fiber membranes also yielded similar results, where the CO₂ mass transfer rates were significantly increased. An increase in the number of CA layers coated onto the membrane corresponded with an increased mass transfer coefficient in the transport of CO₂ across the hollow fiber membranes, as did a reduced adsorption flow rate or an increased polyelectrolyte adsorption contact time. Both porous polypropylene (PP) and nonporous polydimethoxysilane (PDMS) membranes were tested in contactor operations. The degree of pore wetting on the PP was also investigated to determine its effects on the overall rates of mass transfer.
Any feasibility study regarding the use of the enzymes always has a concern regarding the operational lifespan of the enzyme. Nonporous PDMS hollow fiber membranes were used to separate the effects of pore wetting from enzyme deactivation in monitoring the decline of the mass transfer coefficient over time. The hollow fibers were operated at elevated temperatures of 35°C and 50°C to determine the survivability of the CA in the membrane contactor at those temperatures. The CA was found to be completely deactivated after 80 days of exposure to 30 wt% K₂CO₃ at 25°C, while its deactivation was complete after only 3 days of exposure to 30 wt% K₂CO₃ at 50°C.

The immobilized CA was also contacted with toxic gases such as nitric oxide (NO) and sulfur dioxide (SO₂) or their associated nitrate (NO₃⁻) and sulfate (SO₄²⁻) ions in solution, as these are components of post-combustion gas streams that can inhibit the activity of the CA. The exposure of the immobilized CA to the dry gases or their associated anions did not significantly affect the activity of the immobilized CA.

It can therefore be said that CA is a useful promoter for the absorption of CO₂ over a short term period. However, its stability under harsh operating conditions has to be improved for further long-term operational feasibility.
Declaration

This declaration is to certify that

(i) The thesis comprises only my original work towards the degree of Doctor of Philosophy except where indicated in the Preface.

(ii) Due acknowledgement has been made in the text to all other material used.

(iii) The thesis is fewer than 100000 words in length, exclusive of tables, maps, bibliographies and appendices.

Joel Kah Jin Yong
18 August 2016
Preface

Sections of this thesis have been published as the following journal articles:


Note: Further journal papers presenting the remainder of the work in this thesis are currently in preparation.

Sections of this thesis have also been used for poster presentations at the following conferences:


2) J. K. J. Yong, F. Caruso, G. W. Stevens, S. E. Kentish, The Fabrication of Ultrathin Films Containing Carbonic Anhydrase on
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</tr>
<tr>
<td>--------</td>
<td>----------------------------------</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>Number of hollow fiber membranes</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>Number of overtones</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>Gas pressure</td>
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</tr>
<tr>
<td>Per</td>
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</tr>
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</tr>
<tr>
<td>Sc</td>
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<tr>
<td>Sh</td>
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</tr>
<tr>
<td>t</td>
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</tr>
<tr>
<td>T</td>
<td>Absolute temperature</td>
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</tr>
<tr>
<td>v</td>
<td>Liquid flow velocity</td>
<td></td>
</tr>
<tr>
<td>x0</td>
<td>Solvent loading of CO₂</td>
<td></td>
</tr>
<tr>
<td>z</td>
<td>Charge number</td>
<td></td>
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<tr>
<td></td>
<td>Dimensionless</td>
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**Subscripts**

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<thead>
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<tr>
<td>eff</td>
<td>Effective</td>
</tr>
<tr>
<td>D</td>
<td>Decay/Deactivation</td>
</tr>
<tr>
<td>f</td>
<td>Film</td>
</tr>
<tr>
<td>G</td>
<td>Gas phase</td>
</tr>
<tr>
<td>H</td>
<td>Hydraulic</td>
</tr>
<tr>
<td>i</td>
<td>The i&lt;sup&gt;th&lt;/sup&gt; species</td>
</tr>
<tr>
<td>K</td>
<td>Knudsen</td>
</tr>
<tr>
<td>L</td>
<td>Liquid phase</td>
</tr>
<tr>
<td>m</td>
<td>Membrane</td>
</tr>
<tr>
<td>nw</td>
<td>Non-wetted</td>
</tr>
<tr>
<td>out</td>
<td>Outer membrane</td>
</tr>
<tr>
<td>p</td>
<td>Pore</td>
</tr>
<tr>
<td>q</td>
<td>Quartz</td>
</tr>
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</table>
sl       Solid-liquid interface  

w       Wetted  

### Greek Letters

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<thead>
<tr>
<th>Term</th>
<th>Symbolizes</th>
<th>Units</th>
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<td>Pore characteristic</td>
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<td>γ</td>
<td>Surface tension</td>
<td>N m⁻¹</td>
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<td>δ</td>
<td>Membrane thickness</td>
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<tr>
<td>Δ</td>
<td>Change</td>
<td>Dimensionless</td>
</tr>
<tr>
<td>ε</td>
<td>Membrane porosity</td>
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</tr>
<tr>
<td>ϕ</td>
<td>Packing factor</td>
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<tr>
<td>ρ</td>
<td>Density</td>
<td>kg m⁻³</td>
</tr>
<tr>
<td>η</td>
<td>Viscosity</td>
<td>Pa s</td>
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<td>λ</td>
<td>Decay constant</td>
<td>s⁻¹</td>
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<tr>
<td>μ</td>
<td>Shear modulus</td>
<td>Pa</td>
</tr>
<tr>
<td>τ</td>
<td>Membrane tortuosity</td>
<td>Dimensionless</td>
</tr>
<tr>
<td>θ</td>
<td>Contact angle</td>
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</tbody>
</table>
Chapter 1: Introduction

The emissions of carbon dioxide (CO₂) into the atmosphere is of great concern today because these create environmental issues that are associated with global warming [1]. CO₂ is a greenhouse gas that traps infrared radiation from the sun within the atmosphere and prevents it from escaping, which ultimately causes the Earth to become warmer over time. There has been an increase in CO₂ emissions that are mainly derived from human industrial activities [2], such as thermoelectric power plants, cement plants and steel plants. A report from the European Commission estimated the quantity of CO₂ emitted into the atmosphere to be approximately 34 billion tons in 2011 [3], with the trend set to increase over the next few decades. These CO₂ emissions have caused problems with weather patterns around the world, including extreme weather conditions, the melting of the polar ice caps and the flooding of low-lying islands and territories. CO₂ functions as a greenhouse gas (GHG) that traps infra-red radiation within the atmosphere, which prevents the dissipation of heat energy away from the planet, hence causing small but gradual increases in the overall global temperature [4]. These emissions must therefore be managed to maintain a sustainable living environment [5].

Many attempts have been made to reduce the CO₂ emissions from anthropogenic activities to counteract the undesirable effects of climate change. Carbon capture and storage (CCS) technologies [1] are engineering solutions that target the capture of CO₂ that is produced from major emitters during the combustion of fossil fuels. These technologies include the separation of CO₂ from syngas prior to combustion [6], the removal of CO₂ from flue gases after the combustion of fossil fuels [7-11], or the removal of CO₂ after fuels are combusted in pure oxygen [12]. This captured CO₂ can then be stored underground in geological formations such as deep saline aquifers or disused hydrocarbon reservoirs [13, 14] or precipitated as carbonates that are
environmentally benign [15, 16] to prevent their emission into the atmosphere.

Carbon dioxide is a molecule that is thermodynamically stable and requires much energy for decomposition into its carbon and oxygen constituents. In addition, the concentration of CO$_2$ within typical power station flue gases is only approximately 4-14 vol%, which adds to the difficulty of developing feasible post-combustion CCS strategies [7]. A large variety of approaches to this problem have been reviewed, including the use of physical adsorption [17, 18], membrane gas absorption [19-22], cryogenics [23] and hydrate formation [24, 25]. Algae-based CCS technologies have also been investigated as a promising alternative, with CO$_2$ fixation by the algae being able to generate algal biomass that can then function as an alternative energy source [26]. A list of technologies that have been used or are being explored is shown in Table 1-1 [27].
**Table 1-1.** A technology study conducted by the International Energy Agency in 2008, which summarizes the many CCS technologies that are currently being researched and/or applied on industrial scales. This table was obtained from [27].

<table>
<thead>
<tr>
<th>Capture Method</th>
<th>Post Combustion</th>
<th>Pre-Combustion</th>
<th>Oxyfuel Combustion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Technology</strong></td>
<td><strong>Current</strong></td>
<td><strong>Future</strong></td>
<td><strong>Current</strong></td>
</tr>
<tr>
<td>Membranes</td>
<td>Polymeric</td>
<td>-Ceramic</td>
<td>-Ceramic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Facilitated</td>
<td>-Palladium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Carbon molecular sieve</td>
<td>-Reactors</td>
</tr>
<tr>
<td>Solvents/Absorption</td>
<td>Chemical solvents</td>
<td>-Improved process designs</td>
<td>-Chemical solvents</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Improved solvents</td>
<td>-Improved design</td>
</tr>
<tr>
<td>Cryogenics</td>
<td>Liquefaction</td>
<td>-Hybrid process</td>
<td>-Novel contacting equipment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Anti-sublimation</td>
<td>-Physical Solvents</td>
</tr>
<tr>
<td>Solid Sorbents</td>
<td>Zeolites</td>
<td>-Carbonates</td>
<td>-Zeolites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Carbon based solvents</td>
<td>-Hydrotalcites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Pressure Swing</td>
<td>-Zirconates</td>
</tr>
<tr>
<td>Biotechnology</td>
<td>Algae absorption</td>
<td>-Vacuum Swing</td>
<td>-Pressure Swing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Alumina</td>
</tr>
<tr>
<td></td>
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</tbody>
</table>

Adapting this technology for high pressure
However, CCS technologies such as adsorption remain more useful for the capture of streams containing CO₂ at high partial pressures [18, 28], and are less effective at CO₂ capture from post-combustion flue gas streams. The most effective approach to date for addressing the intransigent problem of reducing the rate of CO₂ emissions from post-combustion gas streams into the atmosphere has focused on the use of chemical absorption of CO₂ by liquid solvents, because CO₂ is an acid gas that reacts readily with amine bases. The solvents that are most commonly used in CCS technologies at the industrial scale are alkanolamines such as 30 wt% monoethanolamine (MEA) or diethanolamine (DEA) [29] which have been well characterized over the years [1, 17, 18, 30]. The use of membrane contactors in membrane gas absorption (MGA) processes has been evaluated as an alternative to the use of absorption columns, where a hydrophobic membrane material is used as a contacting barrier between a CO₂ gas stream and an aqueous solvent stream. The concept of an MGA process is illustrated in Figure 1-1 by Yan et al. [31]:

![Figure 1-1. The operation of an MGA process. This figure was obtained from [31].](image-url)
The CO₂ diffuses across the membrane and is absorbed into the solvent while the solvent is prevented from diffusing across the membrane barrier because the membrane is hydrophobic. The CO₂-rich solvent can then be transferred into a stripper unit to release the CO₂ from the solvent for solvent reuse, and the pure CO₂ can then be stored elsewhere instead of being released into the atmosphere.

The alkanolamines that are currently used in CO₂ absorption processes are extremely effective in forming stable carbamates when reacted with CO₂, leading to a very efficient removal of CO₂ [18, 32-34]. However, the regeneration of the CO₂ from the carbamate species [32] for CO₂ storage and solvent reuse requires a parasitic supply of energy to release the CO₂ during stripping [9, 17, 18, 35, 36]. This high energy requirement for the stripping process accounts for up to 80% of the operating costs of the absorption process [37].

It is possible to use different solvents and additives, such as a combination of hindered and tertiary amines [34], piperazine [37-40], aqueous ammonia [38, 41], amino acids [31, 42-45] and potassium carbonate (K₂CO₃) [6, 46, 47]. The use of these solvents or additives reduces the energy demand for the stripping process and provides an increased CO₂ loading per mole of solvent. Nevertheless, the drawbacks of using these alternative absorbents are the slow absorption kinetics which limits the effectiveness of recovering high purity carbon dioxide within a reasonable column height.

Carbonic anhydrase (CA) is a naturally occurring enzyme that is known to catalyze the conversion of CO₂ into bicarbonate (HCO₃⁻) at extremely high turnover rates, which may be useful for promoting the absorption rates of CO₂ from gas streams when these alternative solvents are used [7].

CA has been immobilized on solid surfaces such as nanoparticles [35, 48-50] and membranes [51] and shown to provide an improvement in CO₂ absorption rates whenever tested against other surfaces that do not contain CA. Enzyme immobilization is useful as it stabilizes the
enzyme against denaturation and therefore helps to maintain its activity over a longer period of time [52-54]. Nevertheless, immobilization may bring about a reduction [54, 55] in the catalytic activity of the enzyme.

In addition, CA is an enzyme that may be prone to denaturation at the high pH and the high temperatures of post-combustion capture processes, and may not be as effective in catalyzing CO₂ hydration under these conditions. The feasibility of operating CA-promoted absorption processes over a long period hinges on the ability of the CA to survive under these operating conditions as well as the cost of enzyme production.

This thesis focuses on the development of CA as a catalyst and its ability to function at industrial operating conditions in a membrane contactor. The research is targeted at developing new membrane contactors for CO₂ absorption by incorporating CA into thin layers on the surface of the membrane to increase the chemical reaction rates. In turn, this will increase the efficiency in the mass transfer of the CO₂ across the membrane from the gas phase into the liquid phase and thus improve the viability of CO₂ capture using alternative solvents.
Chapter 2: Literature Review

2.1 Introduction

This chapter first provides an overview of current column-based industrial absorption processes with monoethanolamine (MEA) as a solvent before proceeding to investigate the different solvents that are being used for CO₂ absorption. A comparison between membrane gas absorption (MGA) and column absorption processes is used to understand why MGA operations may be more promising than column absorption processes in the years to come. A review on the mass transfer and a characterization of the wetting processes occurring in MGA operations is also provided as a way to understand the CO₂ capture process.

The use of CA as a promoter for increasing CO₂ hydration rates is investigated. Current research using CA in absorption processes is also highlighted in this chapter. Finally, mechanisms for binding CA onto the membrane surface are reviewed.

2.2 Current Solvent Absorption Processes for Carbon Dioxide Capture

Many different types of technologies have been developed for CCS processes, of which the most commonly used is column-based solvent absorption. In this process, flue gas is fed to the bottom of a tall column for it to travel upwards to the top of the column. The gas stream is contacted with a liquid solvent stream that flows from the top of the column to the bottom of the column. The absorption of CO₂ is usually conducted at temperatures of 30-50°C [35, 55]. Packing media are placed within the column to promote contact between the gas and the solvent for more effective absorption. The CO₂-rich solvent is then sent to a regenerator that is operated at 120-140°C [18] to recover the CO₂ for purification and storage, while the stripped solvent is recirculated back into the absorber. The recovered CO₂ can be stored as solid carbonates [16, 56] or pumped into oil wells for enhanced oil recovery.
processes [57]. A schematic of the CO$_2$ absorption/desorption process is shown in Figure 2-1:

![Figure 2-1. Schematic of a solvent absorption process, based on a solvent such as monoethanolamine (MEA).](image)

The absorption of CO$_2$ into MEA forms zwitterionic carbamate species. This is known to be an exothermic reaction that liberates 72 kJ of heat per mole of CO$_2$ that is absorbed. This absorption process follows the chemical reaction as shown in Equation 2-1, resulting in a maximum CO$_2$ loading of 0.5 mole CO$_2$ per mole of MEA [30, 36]:

$$2 MEA + CO_2 \rightarrow MEA^- + H^+ + MEA^-$$ \hspace{1cm} (2-1)

The second-order reaction rate constants observed by various researchers for the reaction of CO$_2$ with MEA are approximately 4-8 m$^3$ mol$^{-1}$ s$^{-1}$, while the rate constant for CO$_2$ with diethanolamine (DEA) is 0.5-3.2 m$^3$ mol$^{-1}$ s$^{-1}$ [58]. The carbamates that are formed are highly stable thermally, which poses a challenge for reversing the reaction [11, 24]. Much energy is required to decompose the carbamate back into MEA and CO$_2$, of the order of 165 kJ per mole of CO$_2$ that is evolved [36].

While MEA is still the most commonly considered solvent for the post-combustion capture of CO$_2$, it is also a highly corrosive substance [1, 7, 59] that readily undergoes degradation in the presence of oxygen [18, 30, 33] and emits harmful volatile organic compounds (VOCs) [30]. Furthermore, its high relative volatility also results in solvent loss and
means that frequent replenishment of the solvent is necessary [60]. MEA solvent replenishment has been estimated to be approximately 10% of the total operating costs [61].

Bello and Idem [62] summarized a range of MEA degradation products that were produced under different reaction pathways when 5 M MEA was heated at 120°C for 5h, showing the formation of multiple toxic substances as well as other substances that could cause machinery corrosion. In addition, the carbamates that are produced possess a tendency to polymerize at 100-150°C [63], and these polymerized products are able to act as strong chelators that further facilitate machinery corrosion rates [64].

2.3 Alternative Solvents for CO₂ Absorption Processes

Many alternative solvents have been investigated for their CO₂ absorption capabilities, including sterically hindered amines (Section 2.3.1), ammonia (Section 2.3.2), amino acids (Section 2.3.3) and carbonate-based solvents (Section 2.3.4). Most of these solvents face issues with slow absorption kinetics, which therefore necessitates the addition of a promoter or catalyst to the solvent to increase the absorption rate [35, 46, 65]. A wide range of materials that can be used as promoters for aqueous solvent absorption has been investigated, including MEA, piperazine [37-40], amino acids [31, 42-45], boric acid [46] and arsenic acid [66]. A range of promoters for CO₂ absorption into potassium carbonate has been reviewed by Borhani et al. [67]. As an example, MEA has been used as a promoter in K₂CO₃ solvent absorption, where the addition of 5 wt% or 10 wt% MEA to a 30 wt% K₂CO₃ solvent at an operating temperature of 63°C increased the CO₂ absorption rates by factors of 16 and 45, respectively [68]. However, the absorption rate of CO₂ into 30 wt% MEA was found to be increased by a factor of 82 at 35°C [6], which is still significantly higher than the absorption rates provided by the promoted K₂CO₃ solvent.
These promoters should ideally be of low cost, have a CO$_2$ absorption capacity of their own, possess a low vapor pressure to minimize losses to the environment and not be chemically reactive with materials of construction.

2.3.1 Alternative Solvents for CO$_2$ Absorption – Sterically Hindered or Tertiary Amines

Sterically hindered amines such as 2-amino-2-methyl-1-propanol (AMP) and tertiary amines such as N-methyldiethanolamine (MDEA) possess bulky side functional groups that restrict carbamate formation. These amines require much less energy for regeneration [69] and have a higher CO$_2$ capacity than MEA because they react with CO$_2$ at a 1:1 stoichiometry to form bicarbonates instead of the 2:1 stoichiometry of MEA. The reaction mechanism for tertiary amines is shown in Figure 2-2 [11, 30]:

$$
\begin{align*}
R_1 &\quad N-R_3 + CO_2 + H_2O \leftrightarrow R_1 \quad NH^+ + HCO_3^- \\
R_2 &\quad R_2 R_3
\end{align*}
$$

Figure 2-2. Reaction mechanism for tertiary amines reacting with CO$_2$. This figure was obtained from [11, 30].

These solvents are known to be less volatile than MEA, which also limits the rate of solvent loss during the operation [70]. However, their reaction rates are slow relative to the reaction rates that are exhibited by MEA [30, 34, 69, 71]. At room temperature, the second order reaction rate constant for the reaction of MDEA with CO$_2$ is approximately 3 orders of magnitude lower than the rate constants exhibited by MEA or DEA [58]. Hence, it is necessary to add suitable promoters such as piperazine to MDEA and other tertiary amine systems to increase the overall chemical reaction rate [39, 40, 72].

2.3.2 Alternative Solvents for CO$_2$ Absorption – Ammonia

Aqueous ammonia (NH$_3$) has also been reviewed as a solvent for the absorption of CO$_2$, having demonstrated a noticeably lower
operating energy requirement than the alkanolamines [73]. The reaction between ammonia and CO$_2$ follows a single-step termolecular mechanism with a basic molecule B, which is illustrated in Figure 2-3 [41]:

![Reaction mechanism for the reaction of ammonia with CO$_2$. This figure was obtained from [41].](image)

It can be observed from Figure 2-3 that the lone pair of electrons on the N atom attacks the electron-deficient carbon atom on CO$_2$. A base species B then attacks the NH$_3$ molecule and extracts a hydrogen atom to form a BH$^+$ species and a carbamate species (NH$_2$COO$^-$). The carbamate that is formed decomposes rapidly into ammonium bicarbonate (NH$_4$HCO$_3$) as it is more unstable than the carbamate formed from the reaction with MEA [74]. The ammonium bicarbonate can then be readily precipitated as a solid material at ambient temperatures [75, 76] and used as soil fertilizers [73] to minimize the overall production of waste, or it can be thermally decomposed at 58°C into its NH$_3$ and CO$_2$ constituents for the purpose of solvent reuse [75].

The reaction kinetics of NH$_3$ with CO$_2$ is significantly slower (by approximately 4 to 10 times) than that of MEA [41], though there is no evidence of any accumulation of ammonium carbamate within the reaction system because of its instability, which indicates that NH$_3$ has a good cycling capacity for CO$_2$ absorption [74].

**2.3.3 Alternative Solvents for CO$_2$ Absorption – Amino Acids**

Amino acids are known to react with CO$_2$ in the same way as MEA with their free –NH$_2$ functional group, as is illustrated in Figure 2-4 for the potassium salt of an amino acid [77, 78]:

![Reaction mechanism for the reaction of amino acids with CO$_2$. This figure was obtained from [77, 78].](image)
Figure 2-4. Reaction mechanism of amino acid salts with CO₂. This figure was obtained from [77, 78].

The CO₂ reacts with the amine group on the amino acid to form a zwitterion that is then subsequently deprotonated by a base B. The mass transfer rates of CO₂ into potassium glycinate has also been shown to be similar to MEA, though the CO₂ absorption capacity of the potassium glycinate decreases at higher temperatures, possibly because of a reduced CO₂ solubility [31]. Song et al. [43] characterized the suitability of 16 different amino acid salts and concluded that the addition of piperazine to some of those salts boosted the cycling capacity of the absorption solvent, though the low solubilities of some of these salts resulted in downstream precipitation.

2.3.4 Alternative Solvents for CO₂ Absorption – Carbonate-Based Solvents

The absorption of CO₂ into K₂CO₃ is known to form potassium bicarbonate (KHCO₃) as shown in Equation 2-2 [46]:

\[
CO_2 + K_2CO_3 + H_2O \rightarrow 2KHCO_3
\] (2-2)

The absorption exhibits similar CO₂ fluxes to the physical absorption of CO₂ into water at room temperature [6], which indicates that the CO₂-K₂CO₃ system has to be operated at elevated temperature [66] or that a promoter has to be used. Hot potassium carbonate solutions (20-40 wt% K₂CO₃) [67] have been used at industrial scale for CO₂ absorption in the Benfield process [65, 79]. Again, there is significant evidence that the operating energy requirement is lower than
that of alkanolamine absorption [9] because the HCO₃⁻ ions that are produced upon the absorption of CO₂ into K₂CO₃ require less energy for regeneration than the carbamates produced from the alkanolamines [46, 80]. The absorption enthalpy of CO₂ in K₂CO₃ is only 37% that of the absorption enthalpy for CO₂ in amine systems [79].

In addition, K₂CO₃ does not possess the reactive organic functional groups that the alkanolamines possess. These functional groups have a higher propensity to be oxidized or polymerized during the process of alkanolamine degradation upon exposure to heat. This relative chemical inertness allows for a better K₂CO₃ cycling capability. Both the solvent (K₂CO₃) and the product (KHCO₃) are non-volatile, in comparison to other organic solvents such as the alkanolamines or ammonia. KHCO₃ is known to liberate CO₂ upon heating, resulting in the evolution of CO₂ and the regeneration of the K₂CO₃.

Sodium carbonate (Na₂CO₃) systems have been considered for similar reasons [81], though it has to be noted that Na₂CO₃ has a lower saturation solubility in water than K₂CO₃ [82], which results in K₂CO₃ possessing a higher CO₂ loading capacity than Na₂CO₃. However, at high CO₂ solvent loadings in K₂CO₃, there may be a greater tendency for the precipitation of potassium bicarbonate (KHCO₃) to occur, which is a problem that the alkanolamine absorption systems do not demonstrate.

2.4 Membrane Gas Absorption for Carbon Dioxide Capture
2.4.1 The Use of Membrane Contactors for Membrane Gas Absorption

The drawbacks of the use of absorption columns such as those shown in Figure 2-1 include the high capital cost and the requirement for a large footprint for placing these columns [18, 83, 84]. More recently, the use of membrane contactors in gas absorption has been investigated, as these can have a larger surface-to-volume ratio than packed columns [85, 86], resulting in a smaller footprint and a larger mass transfer coefficient than a traditional packed column [84, 87, 88].
Therefore, the use of membrane contactors appears to be a promising substitute to the traditional column absorption methods that are currently being used.

The pioneers of using porous hollow fiber membranes as contactors for MGA processes were Cussler and his co-workers [85, 86, 89], who modelled the transport of various gases through polypropylene (PP) hollow fibers into an absorbing solvent as alternatives to packed column absorption systems.

**Figure 2-5.** An illustration of the MGA process where solvent is pumped through the shell side and gas is pumped through the lumen. This figure was obtained from [6].

In an MGA process (shown in Figure 2-5), the solvent flows through the shell side of a hollow fiber membrane bundle, while the flue gas moves through the bore or the lumen side of each fiber. The gas diffuses across the membrane and is absorbed into the liquid, while the membrane acts as a barrier that prevents the liquid stream from entering the gas stream. The membrane is hydrophobic in nature, which prevents aqueous solvents from easily permeating through it. However, the membrane itself is also microporous to allow for easy gas diffusion.
The separation of CO₂ from the other components within the post-combustion flue gas is achieved by developing a chemical potential gradient across the membrane based on the concentration difference between the CO₂ in the gas phase and the CO₂ in the liquid phase. This concentration difference acts as the driving force for the mass transfer of the material across the membrane from the feed stream into the product stream for removal, hence achieving a separation of one component from the others.

The solvent is generally pumped through on the shell side of the fibers while the gas is fed through the lumen of the fibers. This reflects the fact that most microporous membranes have a ‘skin’ layer on the outside surface that has a smaller pore size and thus reduces liquid penetration. It is also possible for the solvent to be pumped through the lumen side of the fiber and the gas on the shell side. A separate hollow fiber membrane contactor can be used for the stripping operation [90].

It has to be noted in MGA operations that the membrane itself does not provide any form of gas selectivity because of its high porosity, but the solvent is the key to maintaining a good gas separation [42]. Solvents that provide a basis for a chemical reaction as well as a physical absorption are preferred over solvents that are used purely for physical absorption because they provide higher gas absorption capacities and faster mass transfer rates. However, physical absorption may be more cost efficient in cases where the gas stream is operated at much higher CO₂ partial pressures.

Falk-Pedersen et al. [84] highlighted the benefits of using a membrane contactor for CO₂ capture on an offshore platform, as is listed in Table 2-1:

<table>
<thead>
<tr>
<th>Design Parameter</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating Cost</td>
<td>38-42</td>
</tr>
</tbody>
</table>

Table 2-1. A comparison of using a membrane contactor with an absorption column for CO₂ absorption offshore [84]
Table 2-1 indicates that there is a significant reduction in the operating and capital costs, as well as the area that is required to install the membrane contactor equipment. There is also a reduced operating weight, which is more useful for consideration when installing these units on an offshore platform. These cost and area reductions are a result of requiring less material for construction because of the larger surface-to-volume ratio of a membrane contactor in comparison with an absorption column, which then allows for a better mass transfer performance than the column [86, 87, 91-93]. Furthermore, the modularity of the membrane units also allows for a linear scale up process [92, 94].

2.4.2 Membrane Materials for Hollow Fiber Membrane Contactors

Common hydrophobic materials that are used as membrane contactors include PP [6, 31, 83, 85-87, 91, 95, 96], polyvinylidene difluoride (PVDF) [97-99] and polytetrafluoroethylene (PTFE) [6, 83, 87]. A polyether ether ketone (PEEK) membrane material has also been modified to become superhydrophobic and investigated for post-combustion CO₂ capture [9]. Asymmetric polysulfone hollow fiber membranes have also been fabricated for investigating CO₂ absorption into water [100]. These membrane contactors are used over a wide range of gas-liquid separation applications, including blood treatment [51], gas-liquid absorption or stripping operations [92] and membrane distillation [101].
PP is the most common type of membrane material that is used in membrane contactors; however, an untreated PP membrane changes its structure upon prolonged exposure to MEA [88], resulting in a reduced mass transfer coefficient through pore wetting [83, 87, 96]. PTFE is a more hydrophobic material that is more chemically inert than PP and may therefore be more useful as a membrane contactor material. However, PTFE also experiences morphological changes upon prolonged exposure to MEA, though the changes are not as significant as that observed for PP [88]. Furthermore, PTFE is a much more expensive material than PP, and this cost negates the capital savings of a traditional membrane contactor over an absorption column [84, 88]. Yan et al. [102] have determined that MGA absorption processes are more economically efficient than column absorption processes in China if the membrane price can be kept at less than RMB70/m² (approximately AUD15/m²) and if the membrane can maintain its mass transfer performance over a period of 5 years (at 2008 prices). Nevertheless, most untreated PP membranes face issues with pore wetting and an alteration in their morphological structure when operated with MEA as the absorption solvent, which will reduce the overall membrane lifespan.

2.5 Membrane Mass Transfer in Hollow Fiber Membrane Contactors

2.5.1 Elucidating the Overall Mass Transfer Coefficient

The mass transfer of CO₂ across a microporous hollow fiber membrane comprises 3 steps: the gas phase mass transfer from the bulk gas phase to the gas-membrane interface, the diffusion across the membrane, and the liquid phase mass transfer (including physical absorption and chemical absorption) from the membrane-liquid interface to the bulk liquid phase.

Overall, the CO₂ flux (N_{CO₂}) when pure CO₂ is absorbed is related to the concentration difference between the gas concentration at the
membrane-gas interface \((C_G)\) and the theoretical gas phase \(\text{CO}_2\) concentration that is in equilibrium with the \(\text{CO}_2\) concentration in the bulk liquid phase \((C_G^*)\), such that:

\[
N_{\text{CO}_2} = K(C_G - C_G^*)
\]  

(2-3)

When the concentration of dissolved \(\text{CO}_2\) in the liquid is small, as it is in the case of gaseous \(\text{CO}_2\) dissolving in an aqueous solvent, \(C_G^*\) is small relative to \(C_G\) and can therefore be eliminated from Equation 2-3. The overall mass transfer coefficient can then be related directly to the feed gas pressure \(P\) based on the ideal gas law. To express \(K\) in terms of \((\text{m s}^{-1})\), the equation factors in the gas constant, the temperature and the pressure of the system:

\[
K = \frac{N_{\text{CO}_2} \times RT}{P}
\]  

(2-4)

It is possible to determine the \(\text{CO}_2\) flux either by gas phase analysis of the inlet and outlet \(\text{CO}_2\) concentrations [6], or by a titration-based liquid phase analysis of the inlet and outlet \(\text{HCO}_3^-\) concentrations in the solvent [103].

The overall mass transfer coefficient \(K\) for the chemical absorption of \(\text{CO}_2\) can then be written as a function of the liquid phase mass transfer coefficient (in the absence of any chemical reaction) \(k_{lo}\), the gas phase mass transfer coefficient \(k_G\) and the membrane mass transfer coefficient \(k_m\) [6, 22, 93, 104, 105]:

\[
\frac{1}{K} = \frac{1}{k_G} + \frac{1}{k_m} + \frac{1}{mE k_{lo}}
\]  

(2-5)

where \(m\) is a dimensionless Henry’s Law constant for \(\text{CO}_2\) solubility within the solvent and \(E\) is the enhancement factor that quantifies the total flux of gas absorbed into a reactive solvent relative to the flux of gas that is absorbed by physical absorption with a non-reactive solvent.

The value of \(E\) can be determined from the Hatta number \((Ha)\) as per Equation 2-6. \(Ha\) relates the overall absorption rate to the physical absorption rate of \(\text{CO}_2\) and is defined in Equation 2-7 for the absorption of \(\text{CO}_2\) into \(\text{K}_2\text{CO}_3\) [106]:
where $k$ is the second order chemical reaction rate constant between CO$_2$ and hydroxyl ions, $D_L$ is the diffusivity of CO$_2$ in the liquid solvent, $K_e$ is the reaction equilibrium constant and $x_0$ is the solvent loading of CO$_2$, which is the ratio of HCO$_3^-$ ions to K$^+$ ions in the solvent. The value of $K_e$ can be determined as a function of temperature using a correlation provided by Savage et al. [106] in Figure 2-6:

\[ K_e = \frac{kD_LK_e^0}{k_L^0} \left( 1 - \frac{x_0}{2x_0} \right) \]

\[ E = \frac{Ha}{tanh (Ha)} \]

\[ Ha = \sqrt{\frac{kD_LK_e^0}{k_L^0}} \]

**Figure 2-6.** The dependence of $K_e$ on temperature. This figure was obtained from [106].

The determination of the reaction rate constant ($k$) for CO$_2$ in K$_2$CO$_3$ has been found to follow a correlation involving temperature ($T$) and ionic strength ($I$), as shown in Equation 2-8 [107]:

\[ \log k = 13.635 - \frac{2895}{T} + 0.08I \]
Other correlations for determining the $\text{CO}_2$-$\text{K}_2\text{CO}_3$ reaction kinetics have been developed with the use of wetted wall columns [46, 77, 108] or stirred tank reactors [109], which are also useful for evaluating the reaction kinetics at elevated temperatures.

The ionic strength ($I$) of a non-ideal solution is dependent on the molality of the $i$th ionic species ($b_i$) and the charge number ($z_i$) of the ionic species that are present in solution. This relationship is shown in Equation 2-9:

$$I = \frac{1}{2} \sum_{i}^n b_i z_i^2$$  

(2-9)

The value of $E$ can then be determined to be approximately 1.2 [6] based on Equations 2-7 to 2-9 when 30 wt% $\text{K}_2\text{CO}_3$ is used as the solvent for absorbing $\text{CO}_2$ at 35°C. In comparison, the value of $E$ for 30 wt% MEA at 35°C is 82, indicating that the chemical reaction in $\text{K}_2\text{CO}_3$ is much slower than the chemical reaction in MEA [6].

At room temperatures, there is essentially no difference in flux when $\text{CO}_2$ is chemically absorbed into $\text{K}_2\text{CO}_3$ or physically absorbed into water [66]. The low $E$ value for the $\text{CO}_2$-$\text{K}_2\text{CO}_3$ system means that the liquid-side mass transfer rate is likely to be a major determinant of the overall mass transfer rate of $\text{CO}_2$.

A summary of $\text{CO}_2$ mass transfer coefficients is outlined in Table 2-2:

<table>
<thead>
<tr>
<th>Membrane Material</th>
<th>Membrane Area (m²)</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Overall Mass Transfer Coefficient ($\times 10^7$ m s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>0.159</td>
<td>30 wt% $\text{K}_2\text{CO}_3$</td>
<td>35-65</td>
<td>4 – 11 [6]</td>
</tr>
<tr>
<td>PTFE</td>
<td>0.014</td>
<td>30 wt% $\text{K}_2\text{CO}_3$</td>
<td>35-65</td>
<td>4 – 10 [6]</td>
</tr>
</tbody>
</table>
2.5.2 The Henry’s Law Constant

Henry’s Law constants take on different units depending upon how they are defined. The most traditional form of Henry’s Law relates the partial pressure of a gas to its equilibrium solubility at the gas-liquid interface when the gas is brought into contact with the liquid:

\[ P_{Gi} = HC_{Li} \]  (2-10)

where \( P_{Gi} \) is the partial pressure of the gas at the interface, \( C_{Li} \) is the concentration of the gas in the liquid at the interface and \( H \) is the Henry’s Law constant (with units of Pa m\(^3\) mol\(^{-1}\)). The version of Henry’s constant that is used in Equation 2-5, \( m \), is dimensionless and can be related to \( H \) as shown in Equation 2-11:

\[ m = \frac{C_{Li}}{C_{Gi}} = \frac{RT}{H} \]  (2-11)

where \( C_{Gi} \) is the gas concentration at the interface, \( R \) is the gas constant and \( T \) is the absolute temperature.
Weisenberger and Schumpe [113] developed an empirical method to estimate the physical solubilities of various gases in different salt solutions. Other researchers further extended this work to estimate the solubility of CO\textsubscript{2} based on the physical solubility of a similar sized gas, dinitrogen monoxide (N\textsubscript{2}O) [82, 109, 114]. The physical solubility of CO\textsubscript{2} in an aqueous solution was found to be approximately 0.73 times that of the physical solubility of N\textsubscript{2}O in the same solution [115]. The values of m for CO\textsubscript{2}-water systems [116] and CO\textsubscript{2}-K\textsubscript{2}CO\textsubscript{3} systems [109] have been tabulated. At higher temperatures, the solubility of CO\textsubscript{2} in K\textsubscript{2}CO\textsubscript{3} can be estimated using a correlation developed by Astarita et al. [107] or by the method proposed by Weisenberger and Schumpe [113].

The correlation prepared by Astarita et al. [107] can be represented as Equation 2-12 for K\textsubscript{2}CO\textsubscript{3} concentrations of up to 1.8 M:

\[
\log \left( \frac{m_{CO_2,K_2CO_3}}{m_{CO_2,H_2O}} \right) = 0.025C_{CO_3}
\]

(2-12)

where C\textsubscript{CO\textsubscript{3}} is the total concentration of carbonate and bicarbonate ions present in solution at temperature of up to 50°C and the value of m for CO\textsubscript{2} in water (m\textsubscript{CO\textsubscript{2},H\textsubscript{2}O}) is approximately 0.83 [116, 117]. Knuutila et al. [82] also presented similar results for higher K\textsubscript{2}CO\textsubscript{3} concentrations of up to 2.8 M.

2.5.3 Liquid Phase Mass Transfer Correlations

The classical Lévêque correlation can be used to predict tube side flow in a hollow fiber module [118], which is illustrated in Equation 2-13:

\[
Sh = 1.62 \ Gz^{0.33}
\]

(2-13)

where Sh is the Sherwood number and Gz is the Graetz number. The limiting condition for Equation 2-13 to be valid is that Gz > 4. Gz is defined in Equation 2-14 and Sh is defined in Equation 2-15:

\[
Gz = \frac{ReScd_H}{L}
\]

(2-14)

Various correlations have also been developed to determine an estimate of the shell side liquid phase mass transfer coefficient in
absorption processes. These correlations relate the Sherwood number (Sh) to the Reynolds number (Re), the Schmidt number (Sc) and the packing density (ϕ) [119]:

\[ Sh = \frac{k_{L0} d_H}{D} = f(\phi) Re^\alpha Sc^\beta \]  

(2-15)

\[ Re = \frac{\rho v d_H}{\eta} \]  

(2-16)

\[ Sc = \frac{\eta}{\rho D} \]  

(2-17)

where \( k_{L0} \) is the liquid-side mass transfer coefficient without any chemical reaction, \( L \) is the length of the membrane module, \( d_H \) is the hydraulic diameter of the membrane module, \( D \) is the diffusivity of the gas in the liquid, \( \alpha \) and \( \beta \) are regression constants, \( \eta \) is the liquid viscosity, \( v \) is the liquid flow velocity and \( \rho \) is the liquid density. The packing density, \( \phi \), is determined to be the total membrane cross-sectional area divided by the cross-sectional area of the membrane module.

The value of \( d_H \) can be calculated as per Equation 2-18 [119]:

\[ d_H = \frac{4 \times \text{cross-sectional area of flow}}{\text{wetted perimeter}} = \frac{d_{c,in}^2 - nd_{out}^2}{d_{c,in} + nd_{out}} \]  

(2-18)

Where \( d_{c,in} \) refers to the inner diameter of the membrane module, \( d_{out} \) refers to the outer diameter of the hollow fiber membrane and \( n \) is the number of hollow fibers housed within the membrane module. Correlations that have been widely used in MGA operations for flow that is parallel to the fibers are shown in Table 2-3:

**Table 2-3.** Common shell-side mass transfer correlations for membrane contactors

<table>
<thead>
<tr>
<th>Flow Characteristics</th>
<th>Correlation</th>
<th>Limiting Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube side [118]</td>
<td>( Sh = 1.62 \ Gz^{0.33} )</td>
<td>( Gz &gt; 4 )</td>
</tr>
<tr>
<td>Shell side [89]</td>
<td>[ Sh = 1.25 \left( \frac{Re_{H}}{L} \right)^{0.93} Sc^{0.33} ]</td>
<td>0.5 &lt; Re &lt; 500 ( \phi = 0.03 )</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Shell side [104]</td>
<td>[ Sh = (0.3045\phi^2 - 0.3421\phi + 0.15)Re^{0.90}Sc^{0.33} ]</td>
<td>32 &lt; Re &lt; 1290; 0.08 &lt; ( \phi &lt; 0.70 )</td>
</tr>
<tr>
<td>Shell side [120]</td>
<td>[ Sh = (0.53 - 0.58\phi)Re^{0.53}Sc^{0.33} ]</td>
<td>21 &lt; Re &lt; 324; 0.32 &lt; ( \phi &lt; 0.76 )</td>
</tr>
<tr>
<td>Shell side [121]</td>
<td>[ Sh = 5.85(1 - \phi) \frac{d_{H}}{L} Re^{0.66}Sc^{0.33} ]</td>
<td>0 &lt; Re &lt; 500; 300 &lt; Sc &lt; 1000; 60 &lt; Re &lt; 1200; 0.20 &lt; ( \phi &lt; 0.50 )</td>
</tr>
<tr>
<td>Shell side [122]</td>
<td>[ Sh = \frac{1}{0.86 - 0.3\phi} Gz^{0.14+0.3\phi} ]</td>
<td>0 &lt; Re &lt; 100; 0.30 &lt; ( \phi &lt; 0.70 )</td>
</tr>
<tr>
<td>Shell side [123]</td>
<td>[ Sh = (0.52 - 0.64\phi)Re^{0.36+0.3\phi}Sc^{0.33} ]</td>
<td>0.1 &lt; Re &lt; 10</td>
</tr>
<tr>
<td>Shell side [124]</td>
<td>[ Sh = 0.8Re^{0.47}Sc^{0.33} ]</td>
<td>Gz &lt; 60 Closely packed fibers</td>
</tr>
<tr>
<td>Shell side [125]</td>
<td>[ Sh = 0.019Gz ]</td>
<td></td>
</tr>
</tbody>
</table>

Comprehensive reviews of other shell-side liquid phase mass transfer correlations that take into account factors such as the flow orientation have been published elsewhere [92, 119, 126].

In non-wetted membrane pores where the pore diameter is much larger than the size of the gas molecule, diffusion of the gas through the pores is unhindered. In such situations, the gas molecules collide more frequently among themselves than with the membrane pore walls. However, if the pore diameter is smaller, the gas molecules will collide more frequently with the pore walls than themselves. This is referred to as Knudsen diffusion, where the Knudsen diffusivity of the gas within the membrane, \( D_{K,G} \), will be obtained from Equation 2-19 [105, 127]:

\[
D_{K,G} = \frac{4Q}{3} \sqrt{\frac{8RT}{\pi M_A}} \tag{2-19}
\]

where \( Q \) is a constant that is dependent on the pore geometry, the membrane structure and their interactions with the gas molecules, \( R \) is
the gas constant, $T$ is the absolute temperature and $M_A$ is the molecular weight of the gas. For a membrane with ideal cylindrical pores, however, $Q$ can be replaced by the pore diameter $d_p$, as shown in Equation 2-20:

$$D_{K,G} = \frac{d_p}{3} \sqrt{\frac{8RT}{\pi M_A}}$$  \hspace{1cm} (2-20)

If the diffusion of the gas within the membrane is partially governed by unhindered diffusion and partially by Knudsen diffusion, an effective diffusion coefficient $D_{eff}$ can be calculated and used in Equation 2-21 [93, 105, 127]:

$$\frac{1}{D_{eff}} = \frac{1}{D_G} + \frac{1}{D_{K,G}}$$  \hspace{1cm} (2-21)

An understanding of these diffusion coefficients will provide a clearer insight into the modelling of membrane mass transfer characteristics.

### 2.5.4 Modelling Hollow Fiber Mass Transfer in a Wetted Membrane

Membrane contactor operations are prone to membrane pore wetting over time, where the solvent enters into the membrane pores [42, 95] (See Figure 2-7). Pore wetting is known to cause significant increases in the overall mass transfer resistance [42] and reduce the value of $k_m$. A reduced $k_m$ value will in turn cause a reduction in the overall mass transfer coefficient, $K$, and hence reduce the flux of CO$_2$ across the membrane significantly [6, 128]. Indeed, pore wetting can contribute as much as 30% of the overall mass transfer resistance [22, 128, 129].
Figure 2-7. A membrane contactor operating when (a) the membrane pores are non-wetted, (b) the membrane pores are wetted. This figure was obtained from [128].

As the pores become filled with liquid upon wetting with solvent, Equation 2-5 must be modified to incorporate a wetted membrane mass transfer coefficient $k_{m,w}$, which is a smaller number than the non-wetted membrane mass transfer coefficient $k_m$ that was used in Equation 2-5 (see Equation 2-22):

$$\frac{1}{K} = \frac{1}{k_G} + \frac{1}{k_{m,w}} + \frac{1}{m k_L \sigma} \tag{2-22}$$

The enhancement factor $E$ is moved over into the $k_{m,w}$ term because the chemical reaction now occurs within the membrane pores. The wetting of the membrane can be described by Equation 2-23 [6]:

$$\frac{1}{k_{m,w}} = \frac{\delta_w \tau}{m E D_L \varepsilon} + \frac{\delta_{nw} \tau}{D_G \varepsilon} \tag{2-23}$$

where $\delta_w$ and $\delta_{nw}$ refer to the length of wetted and non-wetted pores, respectively, $D_L$ and $D_G$ are the diffusivities of CO$_2$ in the liquid solvent and in the gas phase, respectively, $\tau$ is the tortuosity of the membrane and $\varepsilon$ is the porosity of the membrane. Equation 2-22 can thus be expanded into Equation 2-24:

$$\frac{1}{K} = \frac{1}{k_G} + \frac{\delta_w \tau}{m E D_L \varepsilon} + \frac{\delta_{nw} \tau}{D_G \varepsilon} + \frac{1}{m k_L \sigma} \tag{2-24}$$

The degree of pore wetting can then be taken to be [6]:

$$\frac{1}{k_{m,w}} = \frac{\delta_w \tau}{m E D_L \varepsilon} + \frac{\delta_{nw} \tau}{D_G \varepsilon} + \frac{1}{m k_L \sigma} \tag{2-24}$$

The degree of pore wetting can then be taken to be [6]:
\[ Pore \text{ wetting} = \frac{\delta_w}{\delta_w + \delta_{nw}} \] (2-25)

It can be seen from Equations 2-23 and 2-24 that \( k_m \) decreases with any increase in \( \delta_w \). Equation 2-23, however, is only valid for a symmetric membrane, where \( \tau \) and \( \varepsilon \) remain invariant along the membrane thickness. The modelling of the membrane mass transfer resistance through an asymmetric membrane, which has a thin dense active layer on top of a porous substructure, is much more complicated because of the differences in the tortuosities and the porosities between the active layer and the substructure.

2.6 Membrane Wetting

2.6.1 The Influence of the Contact Angle on Membrane Wetting

One parameter that is commonly used for gauging the wettability of a solid surface is its contact angle \( \theta \) [130], as is depicted in Figure 2-8 [131]:

\[ \gamma_{lv} < \gamma_{sv} < \gamma_{sl} \]

\[ \theta < 90^\circ \quad \theta = 90^\circ \quad \theta > 90^\circ \]

**Figure 2-8.** Illustration of contact angles (\( \theta \)) formed by liquid drops on a solid surface, where \( \gamma_{lv} \) is the surface tension between the liquid and vapor phases and \( \gamma_{sv} \) is that between the solid and the vapor. This figure was obtained from [131].

A surface that has a contact angle of 0-90° is considered hydrophilic, while a contact angle of 90-180° is considered hydrophobic. The surface tension of a liquid is a measure of the energy that is required to increase its surface area. At liquid-gas interfaces, there are larger attractive forces (termed as cohesion) between the molecules in the liquid than those exerted by the gas phase (termed as adhesion).
The net cohesive force is termed the surface tension between the liquid and the vapor phases ($\gamma_{lv}$).

The ability of a membrane to withstand the breakthrough of a liquid through its pores is quantified by a parameter known as the liquid breakthrough pressure (LBP), which is the pressure that is required to force a liquid through the pores of the membrane. Membrane wetting will occur if the feed liquid pressure exceeds that of the membrane LBP [101]. The Laplace-Young equation (Equation 2-26) relates the LBP of a membrane to the contact angle and the pore size of the membrane [31, 93, 96, 130, 132, 133]:

$$LBP = \frac{4\alpha \gamma_{sl} \cos \theta}{d_p}$$ \hspace{1cm} (2-26)

where $\gamma_{sl}$ is the surface tension vector existing between the solid membrane and the liquid solvent (shown in Figure 2-8), $\theta$ is the contact angle that the solvent makes with the membrane and $d_p$ is the maximum pore diameter of the membrane. $\alpha$ is a pore characteristic value, with values of $\alpha = 1$ for cylindrical pores and $0 < \alpha < 1$ for non-cylindrical pores.

For a membrane with a fixed average pore diameter, it is evident that a reduced contact angle causes a reduction in the LBP, which therefore allows the solvent to penetrate into the membrane more easily. A list of membrane-solvent compatibilities was developed by Dindore et al. [93], who showed in a set of experiments across a range of solvents that a decrease in the value of the solvent-membrane surface tension from 33 mN m$^{-1}$ to 30 mN m$^{-1}$ could cause a significant reduction in the LBP from 0.9 bar to 0.1 bar.

The addition of inorganic components into water is known to increase the surface tension of the solution, while the addition of organic components reduces the surface tension [111]. The surface tensions of various CO$_2$ absorption solvents are shown in Figure 2-9, as a function of their LBP for a PTFE membrane with a maximum pore diameter of 3.5 $\mu$m [42]. This figure shows that the relationship between
LBP and the surface tension is only described by the Laplace-Young equation if the surface tension is more than 60 mN m\(^{-1}\) in this case.

**Figure 2-9.** The relationship between the LBP and the surface tension for different CO\(_2\) absorption solvents. The straight line illustrates values calculated by Equation 2-26. DMEA is dimethyl ethanolamine and PT is a potassium salt of taurine. This figure was obtained from [42].

The membrane pore wetting phenomenon in an MGA operation occurs as a result of multiple circumstances during the MGA operation. When MEA is used as an absorption solvent instead of K\(_2\)CO\(_3\) with an untreated PP membrane, the lower surface tension of the MEA relative to K\(_2\)CO\(_3\) [111] is responsible for reducing the LBP of the membrane [95, 128]. This reduced surface tension allows wetting to occur more easily with MEA than with K\(_2\)CO\(_3\), which also causes a reduction in the value of \(k_m\) over time.

### 2.6.2 The Prevention of Membrane Wetting

Equation 2-26 indicates that the LBP is dependent on the liquid surface tension, the membrane contact angle and the membrane pore size. The use of liquids with higher surface tensions and membranes
with larger contact angles or smaller pore sizes can thus result in an elevated membrane LBP. Li and Teo [134] attempted to reduce the pore diameter by adding a thin dense silicone skin on top of a porous polyethersulfone membrane, but they found that the addition of the dense layer caused the value of $k_m$ to increase significantly.

Franco et al. [88, 95] were successful in plasma sputter coating an ultrathin layer of PTFE on top of a native microporous PP membrane to increase the overall contact angle and reduce the wettability of the PP. Simioni et al. [135] were also successful in increasing the LBP of nylon membranes after plasma sputtering with PTFE to increase the membrane contact angle to 151°, which provided a greater cost effectiveness than manufacturing PTFE membranes outright. Kreulen et al. [136] coated microporous PP membranes with a 0.7 µm thick layer of silicone rubber, which did not provide any significant increase to the mass transfer resistance. Chabanon et al. [137] also observed a more stable CO₂ absorption flux over a long period of membrane contactor operation with MEA (approximately 1200 h) when using microporous hollow fiber PP membranes that were coated with a dense layer of Teflon or polymethylpentene, while there was a noticeable flux decline over the same timeframe when the microporous PP membranes were used without any modification.

There is a need, therefore, to fabricate thin films that can maintain or increase the LBP of the membrane while simultaneously being able to minimize any increases in the membrane mass transfer resistance. However, a bigger question remains: is it possible to fabricate a thin film onto a membrane surface to simultaneously reduce the effects of wetting and increase the rate of mass transfer? The possibility of developing catalytic membrane reactors by coating membranes with thin films has been demonstrated previously [138]. It may therefore be useful to develop thin film coatings containing immobilized CO₂ absorption promoters for covering membrane surfaces, which can simultaneously reduce the rate of pore wetting by shrinking
the average pore diameter and also increase the rate of CO₂ absorption by promoting the reaction between CO₂ and a reactive solvent. One of these potential promoters is a naturally occurring enzyme known as carbonic anhydrase (CA) [59, 139, 140].

2.7 An Introduction to Carbonic Anhydrase

2.7.1 The Structure of Carbonic Anhydrase

CA is a metalloenzyme that regulates important biological processes within humans and other living organisms such as the acid-base balance within the blood [141], the photosynthesis mechanism in plants [142] and the carbon concentration mechanism in microorganisms [142]. It is defined by the Enzyme Commission (EC) Number 4.2.1.1 and can be further classified under five different categories (α, β, γ, δ and ζ) based on its structure and its origins [2, 143-146]. The most commonly investigated class of CA is the α form [147], which is generally found in mammals. Figure 2-10 illustrates the structure of α-, β- and γ-CA (shown in Figures 2-10A, 2-10B and 2-10C, respectively). In α-CA, the enzymatic activity is derived from a Zn²⁺ ion that is coordinated to three histidine residues near the center of the molecule in a cone-shaped cavity (Figure 2-10A) [148].

Figure 2-10. Ribbon diagrams of typical α-, β-, and γ-class carbonic anhydrase crystal structures. Active site zinc ions are shown as red space-filled spheres; monomer subunits in the β- and γ-class carbonic anhydrases are individually colored. (a), α-class, human isozyme II, 30 kDa; (b), β-class, Escherichia coli, dimeric unit of the native tetramer, 98.8 kDa; (c), γ-class, Methanobacterium thermophila, 69 kDa. This figure was obtained from [148].
β-CA (Figure 2-10B) predominates in plants and algae and is also found in *Bacteria* and *Archaea*. In this case, the Zn$^{2+}$ ion is co-ordinated to two cysteine residues and one histidine residue on the protein [149]. While α-CA is always present as a monomer, β-CA exists as dimers, tetramers, hexamers, and octamers. Conversely, γ-CA (Figure 2-9C) is mostly present in single celled micro-organisms (*Archaea*) and always exists as a trimer [149]. Iron- and cobalt-substituted forms of this γ-CA exhibit greater CO$_2$ hydration rates than the zinc enzyme [149]. ζ-CA, which is extracted from diatoms, has Cd$^{2+}$ as the metal ion catalyst, reflecting the lack of Zn$^{2+}$ available in a marine environment [150]. It can be said, though, that CA is more efficient at CO$_2$ hydration when the Cd$^{2+}$ ion is replaced with Zn$^{2+}$ [151]. The substitution of Zn$^{2+}$ with Co$^{2+}$ on a bovine CA isozyme yielded similar activity results, showing that Co$^{2+}$ was as effective as Zn$^{2+}$ [152], though Cd$^{2+}$ is still the best ion of choice for maximizing CO$_2$ hydration activities.

2.7.2 Reactions Governing the Mechanism of Carbonic Anhydrase Activity

The reaction mechanism for the hydration of CO$_2$ by α-CA is shown in Figure 2-11, where the Zn$^{2+}$ ion in the middle of the CA enzyme abstracts a proton from a surrounding water molecule to generate a negatively charged hydroxide ion in Step 1 [153, 154]:

\[
\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{HCO}_3^- + \text{H}^+
\]
Figure 2-11. CO$_2$ hydration mechanism of carbonic anhydrase. This figure was obtained from [154].

The proton is shuttled to the imidazole functional group on His-64 before being absorbed into solution, regenerating the imidazole on His-64 for abstracting more protons. The hydroxide ion that is formed from Step 1 then attacks the partially positive carbon atom on a solubilized CO$_2$ molecule in Step 2 to form a HCO$_3^-$ ion [1, 139, 149, 154, 155].

The reaction mechanism for the hydration of CO$_2$ by CA can be summarized in Equations 2-27 and 2-28:

\[
\begin{align*}
Zn^{2+} + H_2O & \rightleftharpoons H^+ + Zn^{2+} - OH^- \\
Zn^{2+} - OH^- + CO_2 & \rightleftharpoons Zn^{2+} + HCO_3^-
\end{align*}
\]  

(2-27)  

(2-28)

The overall chemical reaction rate $r_{CA}$ for this enzyme-facilitated hydration can be approximated by Equation 2-29 [1]:

\[
r_{CA} = \frac{k'}{K_M} [CA][CO_2] - [CO_2]^* 
\]

(2-29)

where $K_M$ refers to the Michaelis constant of the reaction and $k'$ is defined as the turnover number and ranges between $10^4$ and $10^6$ molecules of CO$_2$ per molecule of CA per second, depending on the strain of CA that is being used [2, 139, 147, 149, 156, 157]. The positive
[CO₂] term represents the quantity of CO₂ that is being converted into HCO₃⁻, while the negative [CO₂]* term represents the concentration of HCO₃⁻ that is being converted back into CO₂. At a pH that is greater than 7, the forward reaction of Equation 2-24 is favored, leading to the hydration of CO₂ to HCO₃⁻, while the reverse reaction for the dehydration of HCO₃⁻ to CO₂ is favored under acidic conditions [158].

The value of k'/KM (Equation 2-29) is also known as the specificity constant. This value is approximately 1×10⁵ – 4×10⁵ M⁻¹ s⁻¹ for cat-derived CA enzymes [159], while it is reported to be approximately 10⁸ M⁻¹ s⁻¹ for human CA isozymes [153, 157, 160]. Kogut and Rowlett [152] obtained a value of 9×10⁷ M⁻¹ s⁻¹ for bovine CA. Russo et al. [161] obtained a specificity constant of 9.16×10⁶ M⁻¹ s⁻¹ for a thermally stable recombinant carbonic anhydrase at 25°C, while Alper and Deckwer [162] recorded a value of 0.90 L mg⁻¹ s⁻¹ at 25°C with an activation energy of 9.0 kcal mol⁻¹ at pH values ranging from 9.6-11.1. Thee et al. [47] were able to characterize a proprietary thermostable CA strain from Novozymes that exhibited a specificity constant of 9×10⁵ M⁻¹ s⁻¹ in CO₂ hydration.

2.7.3 Characterization of Carbonic Anhydrase Activity

It has been established that the metal ion immobilized within the CA enzyme is the main functional moiety that is responsible for CO₂ hydration [153, 154]. This ion is also capable of functioning as an esterase, such that various researchers have developed a standard assay for quantifying the activity of CA based on the hydrolysis of para-nitrophenyl acetate (p-NPA) [35, 54, 144, 156]. However, the activity of CA in ester hydrolysis may not correlate with the activity of CA in CO₂ hydration and is usually lower in the hydrolysis reaction than it is in the hydration reaction [163].

Different techniques have been formulated for quantifying the activity of CA in CO₂ hydration. Time-based manometric measurements of CA activity were first described by Roughton and Booth [164, 165],
when they compared CO₂ evolution rates in systems with and without CA. Wilbur and Anderson [166] were the first to provide a standardized method by mixing CA and water saturated with CO₂ at 0°C. They determined the time taken for the pH to drop from 8.0 to 6.3 as compared to a blank mixture that contained no CA. The activity of the CA (in terms of Wilbur-Anderson units) was then taken to be as in Equation 2-30 [166]:

\[ 1 \text{ Wilbur-Anderson unit of activity} = \frac{2 (t_0 - t)}{t} \] (2-30)

where \( t_0 \) is the time taken for the pH of the blank to drop from 8.0 to 6.3 and \( t \) is the time taken for the pH of the CA mixture to drop from 8.0 to 6.3.

However, these activity tests were conducted at low temperatures (near freezing point), which may reduce the activity of the enzyme or produce a large variance in the obtained time results based on the mixing of the solutions or the type of apparatus used to mix the solutions [167]. In addition, the activity of CA at these low temperatures would also be vastly different from the CA activity at room temperature.

The rapid activity of the CA also allows for the use of stopped flow spectrophotometry, where a small volume of CO₂-saturated water and a small volume of CA are brought into contact, which can then be used to determine the hydration rate constants via spectrophotometric measurements [153, 168-170]. Mass spectrometry measurements of isotopic ¹⁸O transfer between CO₂ and HCO₃⁻ have also been developed to determine the kinetics of the hydration reaction [170, 171].

Vinoba and co-workers [48, 49] developed a method for quantifying CO₂ hydration activities by precipitating out the HCO₃⁻ ions as insoluble CaCO₃ and then weighing the dried precipitate to back-calculate the quantity of HCO₃⁻ that was produced over a specific time period. This amount of produced HCO₃⁻ was then compared against the quantity of HCO₃⁻ that was produced from a known mass of enzyme.
2.8 Engineering Carbonic Anhydrase for the Absorption of CO₂

If most native CA strains were to be exposed to the operating temperatures within a solvent absorptions process, especially in the stripper stage, they would undergo denaturation extremely readily. Most native CA strains are known to lose their activity at 55-65°C [172-174]. Thermostable CA strains have been found in thermophilic microorganisms [80, 143, 175] and have also been produced in engineered recombinant microorganism strains [176, 177]. These strains are able to tolerate temperatures of 80-90°C. These temperatures are still lower than that observed in a CO₂ stripper, but may permit operation within the CO₂ absorber, at 30-50°C. However, the enzyme may need to be engineered to tolerate the high ionic strength and pH of capture solvents.

2.8.1 Molecules that Mimic CA

An alternative to the genetic engineering of CA strains is the manufacture of artificial enzyme mimics. These mimics generally involve the use of a Zn²⁺ ion bound to various ligands. The most active small molecule CA mimic that has been identified thus far is the 1,4,7,10-tetraazacyclododecane chelate of zinc(II) perchlorate, referred to as “zinc cyclen” (Figure 2-12) [172]. This molecule has a fivefold lower activity than the native bovine CA enzyme on a mass basis, but is commercially available on a large scale [172].

![Figure 2-12](image)

Figure 2-12. Zinc cyclen perchlorate consists of a Zn²⁺ ion bound to a cyclic amine ligand 1,4,7,10-tetraazacyclododecane. This Zn²⁺ ion chelate can act as an effective CA mimic. This figure was obtained from [172].
Furthermore, this mimic molecule has been shown to retain its activity at temperatures of up to 75°C and can retain its structure at temperatures of up to 100°C, suggesting that it can survive the passage through a standard CO₂ stripping cycle for reuse [172]. The catalyst is inhibited by significant bicarbonate concentrations, which drives the reverse reaction in Equation 2-28. However, this inhibition can be overcome by maintaining a high pH (greater than 9) in the absorber, which will minimize the HCO₃⁻ concentration [172]. A range of similar macrocyclic amine-based Zn²⁺ complexes have also been identified as CA mimics that exhibit enhanced CO₂ hydration activities [158, 178-180].

Other Zn²⁺ complexes that have been shown to mimic the action of CA include a zinc-histidine complex [181], a zinc-imidazole complex [7, 182] and a poly(N-vinylimidazole)–zinc (PVI-Zn²⁺) complex [155]. Yao et al. [155] synthesized the PVI-Zn²⁺ complex by first polymerizing N-vinylimidazole through radical polymerization, followed by the mixing of the polymer with zinc acetate for the polymer to capture the Zn²⁺ ions, resulting in the complex that is shown in Figure 2-13:

**Figure 2-13.** The Zn²⁺ ion immobilized within (A) a CA enzyme, (B) a PVI-Zn²⁺ complex. This figure was obtained from [155].
Zastrow et al. [183] constructed an artificial metalloenzyme that included a three-stranded coil containing both a catalytic metal site, \( \text{ZnN}_3\text{O} \) and a separate \( \text{HgS}_3 \) site for structural stabilization. They claimed that this approach provided a simplified construct that retained sufficient complexity to resemble a native protein environment for maintaining the catalytic activity of the CA.

2.9 An Evaluation of Carbonic Anhydrase in Industrial Operations

Much work has been conducted on the effectiveness and energy efficiency of the use of CA for \( \text{CO}_2 \) absorption processes. Arazawa et al. [51] considered the covalent immobilization of CA onto the surface of polymethylpentene membranes in an MGA format to gauge the suitability of the membranes as artificial lungs for removing \( \text{CO}_2 \) from blood and showed that the removal rate of \( \text{CO}_2 \) from the blood was 36% greater for membranes with immobilized CA than for membranes without CA based on hydrolysis activity of \( p\)-nitrophenyl acetate.

Other research has been focused on contained liquid membrane (CLM) contactors. In a CLM setup, a thin film of liquid is immobilized within a microporous membrane. One surface of this membrane is contacted with the feed \( \text{CO}_2 \), so that the \( \text{CO}_2 \) diffuses through the membrane and into the liquid phase [139]. The other surface is contacted with a sweep gas, which draws the dissolved \( \text{CO}_2 \) out of the liquid phase for purification. A CLM thus combines the absorption and desorption process within a single modular setup, which eliminates the need for expensive columns and reduces the overall capital cost. It was established 40 years ago that the presence of solubilized CA within such an immobilized liquid phase enhanced the mass transfer flux of \( \text{CO}_2 \) across the membrane, with a \( \text{CO}_2 \) reaction rate that was proportional to the quantity of CA present within the membrane [184]. More recently, the performance of CA, DEA and sodium bicarbonate (\( \text{NaHCO}_3 \)) for \( \text{CO}_2 \) removal was compared when used in a CLM, with the conclusion that
CA was the most effective at removing CO\textsubscript{2} [33]. The system also demonstrated potential for scaling up to treat industrial flue gases [140].

A variation of the CLM is a hollow fiber contained liquid membrane (HFCLM), where two sets of hollow fibers are packed together into a single microporous hollow fiber permeator. The solvent is contained in the spaces between these fibers (shell side). The flue gas is then passed through the lumen of one set of fibers while the sweep gas is passed through the lumen of the other set. This absorption setup is shown in Figure 2-14 [140]:

Figure 2-14. A conceptual diagram for operation of the Carbozyme Permeator, which uses CA immobilized on the external surface of hollow fiber membranes in a HFCLM format. This figure was obtained from [140].

Borchert and Saunders [176] showed that the efficiency of a HFCLM with a free CA concentration of 0.6 g L\textsuperscript{-1} in a 1 M NaHCO\textsubscript{3} solvent could dramatically improve the absorption rate of CO\textsubscript{2} based on a continuous CO\textsubscript{2} feed concentration of 15%. However, other researchers have shown that a CLM is unable to maintain a long-term stable performance as the free CA that is dissolved in the liquid
denatures and loses its CO₂ hydration activity, and argue that it is more prudent to immobilize the CA within the membrane in a hydrogel phase [154, 185].

In an alternate approach, Yao et al. [155] immobilized a polymeric-metal CA mimic that contained Zn²⁺ ions as a thin polymeric film on a porous support. However, there was no solvent phase in this case. Rather, the humidification of the feed and sweep gas was sufficient to allow the CA mimic to facilitate the transport of the carbon dioxide as it passed across the membrane.

2.10 The Resilience and the Operation Costs of Using Carbonic Anhydrase

2.10.1 The Thermal Stability of Carbonic Anhydrase

An enzyme experiences unfolding upon exposure to heat or harsh pH conditions [186], which is a thermodynamic effect to maintain its stability in its environment. However, the unfolding is usually irreversible and results in the aggregation of these unfolded chains and their subsequent precipitation, leading to an overall loss in enzyme activity.

Therefore, the thermostability of the CA and its resistance to denaturation is an important criterion when using it as a promoter in an absorption operation, especially when considering that the absorption process is conducted at temperatures that are above optimal for mammalian enzymes [175, 187]. This can render common bovine CA or human CA ineffective in conventional CO₂ absorption processes. De Luca et al. [143] have isolated a thermostable CA from Sulfurihydrogenibium yellowstonense, while Borchert and Saunders have obtained a thermostable form of CA from Bacillus clausii [176] and Alvizo et al. [177] have produced a thermostable and salt-stable form of CA from Desulfovibrio vulgaris.
The activity of an enzyme (a) is known to follow an exponential decay mechanism as a function of time (t), according to Equation 2-31 [173, 188-191]:

\[ a = a_0 e^{-\lambda t} \]  

(2-31)

Where \( a_0 \) is the initial enzyme activity at \( t = 0 \) and \( \lambda \) is the decay constant.

It is further known that \( \lambda \) follows an Arrhenius relationship with temperature (T) as shown in Equation 2-32 [173, 188-191]:

\[ \lambda = \lambda_0 e^{\frac{E_D}{RT}} \]  

(2-32)

Where \( \lambda_0 \) is the decay constant at \( T = 0 \) K, \( R \) is the gas constant and \( E_D \) is the deactivation energy. The deactivation energy is approximately 50-150 kcal mol\(^{-1}\) for most enzymes [192], though it may be possible that the immobilization of an enzyme may increase its stability and its resistance to thermal denaturation [52, 189]. Table 2-4 highlights the \( E_D \) values that have been determined by researchers for free and immobilized CA enzymes:

**Table 2-4.** Deactivation energies of carbonic anhydrase enzymes

<table>
<thead>
<tr>
<th>Type of CA</th>
<th>Enzyme Mobility</th>
<th>Method of Determination</th>
<th>( E_D ) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine [173]</td>
<td>Free</td>
<td>( p )-NPA hydrolysis</td>
<td>1075</td>
</tr>
<tr>
<td>Bovine [189]</td>
<td>Free</td>
<td>( p )-NPA hydrolysis</td>
<td>121</td>
</tr>
<tr>
<td>Bovine [189]</td>
<td>Immobilized on polyurethane</td>
<td>( p )-NPA hydrolysis</td>
<td>360</td>
</tr>
<tr>
<td>Human [191]</td>
<td>Free</td>
<td>Differential Scanning Calorimetry</td>
<td>529</td>
</tr>
</tbody>
</table>
The use of enzymes as biocatalysts for industrial operations is highly dependent on the operating costs associated with the production of the enzyme. There can be a high cost associated with the use of enzymes, particularly if they are not reused. As an example, a large quantity of enzyme is required for the pretreatment of lignocellulosic material prior to ethanol production. As the enzyme is not reused, the cost is significant, at USD 0.30 (AUD 0.40) per gallon of ethanol produced (at 2005 prices) [195, 196]. However, the costs can be reduced based on the enzyme yield, the stability of the enzyme and the required enzyme purity, as Migliardini et al. [175] have argued. Enzymes that can be produced at manageable cost have been used in a variety of industrial processes, including detergent production, food production, paper production and in the leather industry [197]. It is shown that the use of enzyme technology is viable in a process when the enzyme production cost is a small fraction of the final product value (0.1-3%) [198].

Furthermore, the denaturation of expensive enzymes and their subsequent replacement with fresh enzymes may become a significant cost issue over a long-term industrial process. Therefore, the CA that is to be used for an industrial carbon dioxide capture process must maintain a certain level of enzymatic activity over long periods of reuse. The process economics of operating a CA-based CO₂ absorption process has not been thoroughly investigated at this point. It can be expected that CA would also contribute a significant cost component towards the operation of CA-facilitated CO₂ absorption processes if it were
consumed during each absorption cycle, rather than if it were continuously reused [199, 200].

2.10.2 Inhibitors of Carbonic Anhydrase Activity

The CA enzyme must also be tolerant to other toxic components that are found within the influent gas feed. The toxic gases that are present in the post-combustion gas streams at coal-fired power plants contain a mixture of sulfur dioxide (SO₂, 40-900 ppm or nitrogen oxides (NO, 100-1500 ppm) [30, 201-203], which are known CA inhibitors.

CA has already demonstrated an inhibited activity in the presence of NOₓ in physiological and pharmacological tests on rabbits [204, 205], which could be a result of the enzyme becoming nitrosylated by the reactive NOₓ molecules or the NOₓ molecules binding to the Zn²⁺ ions that are coordinated to the enzyme molecules [206].

In an MGA process, the toxic gases are able to diffuse into the liquid solvent and react with the solvent to form anions, which then inhibit the hydration of CO₂ (shown in Step 2 of Figure 2-11) from occurring as readily. The activity of CA in the presence of various anions can be modeled as per Equation 2-33 [207]:

\[
Rate\ of\ CO_2\ Hydration = \frac{kC_{enzyme}}{1 + \frac{k}{K_B} \left(1 + \frac{C_{anion}}{K_I}\right)}
\]  

(2-33)

Where k refers to the reaction rate constant, C_{enzyme} and C_{anion} refer to the enzyme and anion concentrations, respectively, K_B is the equilibrium constant governing the deprotonation of the water molecules by the CA (Step 1 in Figure 2-11) and K_I is the inhibition constant. It can be observed from Equation 2-33 that the rate of CO₂ hydration is more severely affected at extremely low K_I values. The K_I values for NO₃⁻ (derived from NOₓ) and SO₄²⁻ (derived from SOₓ) are appreciably large (to the order of 10 mM or greater) with an α-CA derived from *S. yellowstonense* [143] or a γ-CA derived from *Methanosarcina thermophile* [208]. However, it appears that monovalent ions are more likely to cause inhibition than divalent ions in the case of
human [209] or bovine [210] CA. Conversely, high SO$_4^{2-}$ concentrations of up to 1 M were found to enhance the esterase activity of human CA [209].

The activity of bovine CA has been found to be inhibited by a wide variety of other anions [2, 143, 159, 182, 211], including the ions that have already been mentioned previously. However, the inhibition effect of these anions on bovine CA were found to be pH-dependent, where the enzyme activity was found to be dependent on the pH and the type of anions present at low pH (< 7), while the activity was independent of pH at higher pH values [210, 212].

Another common inhibitor to CA activity can be the presence of HCO$_3^-$, present in a K$_2$CO$_3$ solvent as the product from the CO$_2$-OH- neutralization reaction (Equation 2-28). A higher CO$_2$ solvent absorption capacity leads to a higher HCO$_3^-$ concentration, which results in a greater inhibition of the enzyme activity.

Bond et al. [156] showed that there was little indication of inhibition at the concentrations that might be expected in post combustion carbon capture environments for bovine CA (below 100-200 mM of SO$_4^{2-}$ or NO$_3^-$). Similarly, Lu et al. [213] found that anion concentrations of up to 0.9 M SO$_4^{2-}$, 0.2 M NO$_3^-$ and 0.7 M Cl$^-$ did not influence the kinetics of absorption from a CA-loaded potassium carbonate solution. The type of CA that was used in these experiments was undisclosed.

### 2.11 Enzyme Immobilization Techniques

#### 2.11.1 Enzyme Immobilization

The immobilization of an enzyme to a solid substrate is a focal point for research because it aids in the reuse of the enzyme and lengthens its lifespan [52]. Immobilized enzymes do not need to be replaced as frequently as free enzymes, leading to a lower quantity of enzyme required and therefore a lower cost for operating enzyme-based CO$_2$ capture processes. Immobilization has also been shown to improve
the thermostability of various types of enzymes [53, 55, 214, 215], including CA itself [189, 190]. In the MGA process for CO₂ absorption, the immobilization of the enzymes within the absorption stage eliminates the need to expose them to the harsher temperature conditions within the stripper, which also helps to prolong their operational lifespan.

There have been reports of CA enzymes being immobilized onto activated carbon particles [35], silica nanoparticles [48, 55, 216], gold nanoparticles functionalized with silica [49], polyurethane foams [144, 175, 189], chitosan and silica coated magnetic particles [181], alumina [190], chitosan [214] and silica beads [217] for the purpose of CO₂ capture operations. A wide array of methods have been used for immobilization including the use of cross-linked aggregation [48], physical adsorption [48], covalent bonding through reaction with aldehydes [48] or with cyanogen bromide and trimethylamine [51], hydrogels [154, 218, 219], and LbL electrostatic adsorption [220]. A structural analysis of human CA adsorbed onto silica nanoparticles showed that the active site was not significantly disrupted upon adsorption [216], which indicates that most of the enzymatic activity can be preserved after it has been immobilized [48, 49].

It has been shown that the immobilization of the enzyme onto nanoparticles may further enhance the enzyme activity [49], as there is a greater degree of freedom for the enzyme to attach to the curved surfaces of the nanoparticles [221] and hence preserve the active site [222]. However, it is more likely that an immobilized form of CA will demonstrate a decreased specific activity relative to its free form [48, 50, 54, 217]. Immobilization has been shown to improve the thermal stability [35, 50, 189] and the half-life of an enzyme [50, 55, 223], increasing the operating lifespan of the enzyme. Immobilization of enzymes onto nanoparticles can reduce their deactivation rates when subjected to less than optimal operating conditions [224].
The immobilization of CA onto a solid support may be beneficial to maintaining the enzyme activity within a purely liquid phase as measured by $p$-NPA assays [54]. However, the need for the CO$_2$ to migrate through the liquid phase to reach the active site of the enzyme in a gas-liquid absorption process must also be considered. This step can be a rate limiting factor [54, 225] that limits the overall effectiveness of the CA as a promoter [1, 226]. Both Russo et al. [8] and Penders-van Elk et al. [227] have shown that it is more effective to use small particles for the immobilization of CA in a packed column for this reason, as these particles can more readily penetrate the gas-liquid mass transfer boundary layer. Only when the particle size in the resulting slurry reduces below 10 μm in size does the turnover factor approach that of the free enzyme, as shown in Figure 2-15. Other researchers have considered the use of a trickle spray flow over enzyme-immobilized packing [142] to similarly maintain the enzyme close to the gas-liquid interface.

**Figure 2-15.** The turnover factor (ToF) relative to that calculated for free enzyme as a function of the average particle diameter used for enzyme
immobilization. The dashed line is a rough indication of the trend. This figure was obtained from [227].

The immobilization of CA on the surface of a membrane contactor would have a similar effect in maintaining the enzyme close to the gas-liquid interface. However, the immobilization of CA onto membrane supports must be in thin layers to prevent a significant increase in the membrane mass transfer resistance, as shown previously by Li and Teo [134]. The fabrication of submicrometer-thick multilayered enzyme films can simultaneously reduce the distance of the enzyme from the gas-liquid interface while minimizing the membrane mass transfer resistance [101, 228].

2.11.2 Layer-by-Layer Technology

The fabrication of ultrathin multilayered polyelectrolyte films through the electrostatic adsorption of oppositely charged polyelectrolytes has been of great interest since it was first reported [229, 230]. This approach is referred to as the layer-by-layer (LbL) approach and results in ultrathin films at uniform growth rates [231, 232]. The fabrication process occurs by first contacting a surface with a polyelectrolyte solution, followed by a washing step to remove excess polyelectrolyte. Washing of the surface is preferable to drying because drying causes the surface roughness to increase, which in turn causes an increase in the film thickness [233].

An oppositely charged polyelectrolyte solution is then brought into contact with the surface, followed by another washing step. This process is repeated as many times as necessary. A scheme depicting the fabrication of a multilayered enzyme film is shown in Figure 2-16 [234]:
Figure 2-16. Schematic diagram illustrating the alternating assembly of linear polyelectrolytes with globular proteins. A positively charged polion is adsorbed onto a negatively charged substrate surface in Stage 1, followed by the adsorption of negatively charged proteins onto the polion in Stage 2. Stages 3 and 4 illustrate the alternating adsorption of positively charged polion and negatively charged protein to form a thin film on the substrate surface. This figure was obtained from [234].

In Figure 2-16, a positively charged polion is adsorbed onto a negatively charged surface in Step 1 before negatively charged protein globules are introduced to the surface for adsorption in Step 2. Steps 3 and 4 depict alternating repeat adsorbing layers of polion and protein.

The adsorption of a polyelectrolyte chain to an oppositely charged polyelectrolyte chain is caused by an electrostatic attraction of the oppositely charged groups, which then come together for charge neutralization and precipitation to form the film.

During the charge neutralization process, the counterions and the water of hydration are expelled from the solubilized polion chains to cause a spontaneous precipitation of the neutralized polions [235], although this mixing is not thermodynamically ideal [236].

An understanding of the changes in the surface charge as the polyelectrolytes are adsorbed is important in the fabrication of LbL-assembled films, as the ability of an adsorbed polyelectrolyte to completely reverse the surface charge upon adsorption is the key to fabricating uniform films [215, 232, 237-239]. LBL assembly is also dependent on the ionic strength of the polyelectrolyte solutions [240, 241]. A film fabricated from positively charged polyallylamine hydrochloride (PAH) and negatively charged polystyrene sulfonate (PSS)
was found to be smooth, compact and densely packed when the polyelectrolytes were dissolved in water [242]. However, the addition of sodium chloride at increasing ionic strengths caused the polyelectrolytes to coil and form a rougher and more loosely packed film [230, 241, 243]. It can be argued that there exists a critical ionic strength that causes the film structure to change from smooth and dense to rough and loose [244]. Other factors that influence the morphology of the fabricated films include the ionic charge of the polyelectrolytes [245], the concentration of the polyelectrolytes in solution [237] and the isoelectric points (pI) of the polyelectrolytes [246, 247].

There have been a wide range of films that have been fabricated from LbL assembly because of the versatility of the process, its simplicity and the wide range of interactions that the different precursor materials exhibit while they are in contact with and interact with each other, such as covalent interactions, electrostatic interactions, hydrogen bonding and hydrophobic interactions [247-249]. The LbL assembly process is simple and only requires rudimentary laboratory equipment, hence rendering it a relatively inexpensive method in comparison to other techniques, such as the Langmuir-Blodgett technique [249]. LbL films can be fabricated on a wide variety of surfaces, including polystyrene particles [215, 250] and silica nanoparticles [250, 251]; and for many different applications, including light emitting diodes, self-healing conductive surfaces [252], gas sensing [253], immobilized catalysts [231, 234, 246, 254], immunosensors [240] or drug delivery carriers [248, 250, 255]. Of particular relevance to the current thesis, the approach has been used to close up the pores of a microporous or ultrafiltration membrane to form a less porous layer of nanofiltration pore size [223, 224].

Both pure proteins [231, 234, 237, 240, 256, 257] and multicomponent protein films have been immobilized using LbL assembly techniques [234, 246] (Tables 2-5 and 2-6). Table 2-6
illustrates that not all proteins can be assembled into films [246] as they may not possess sufficient charge. Similarly, the protein may form a water soluble complex with the polyelectrolyte used, again preventing the formation of a film [237, 258].
### Table 2-5. A list of proteins that have been immobilized within multilayered polyelectrolyte films using various polyelectrolytes. This table was obtained from [258].

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular weight</th>
<th>Isoelectric point</th>
<th>pH used</th>
<th>Charge</th>
<th>Alternate with</th>
<th>Saturation time (min)</th>
<th>Frequency change of each protein + polyon step (Hz) (±5%)</th>
<th>Thickness of protein + polyon layer (Å) (±10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome</td>
<td>12400</td>
<td>10.1</td>
<td>4.5</td>
<td>+</td>
<td>PSS&lt;sup&gt;-&lt;/sup&gt;</td>
<td>12</td>
<td>150 ± 160</td>
<td>24 ± 16</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>14000</td>
<td>11</td>
<td>4</td>
<td>+</td>
<td>PSS&lt;sup&gt;-&lt;/sup&gt;</td>
<td>16</td>
<td>145 ± 115</td>
<td>23 ± 19</td>
</tr>
<tr>
<td>Histone f3</td>
<td>15300</td>
<td>11</td>
<td>7</td>
<td>+</td>
<td>PSS&lt;sup&gt;-&lt;/sup&gt;</td>
<td>–</td>
<td>140 ± 260</td>
<td>22 ± 42</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17800</td>
<td>7.0</td>
<td>4</td>
<td>+</td>
<td>PSS&lt;sup&gt;-&lt;/sup&gt;</td>
<td>12</td>
<td>250 ± 194</td>
<td>40 ± 31</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>42000</td>
<td>8.8</td>
<td>4.2</td>
<td>+</td>
<td>PSS&lt;sup&gt;-&lt;/sup&gt;</td>
<td>–</td>
<td>For bilayer 220</td>
<td>35</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>64000</td>
<td>6.8</td>
<td>4.5</td>
<td>+</td>
<td>PSS&lt;sup&gt;-&lt;/sup&gt;</td>
<td>16</td>
<td>1100 ± 190</td>
<td>175 ± 31</td>
</tr>
<tr>
<td>Glucoamylase</td>
<td>95000</td>
<td>4.2</td>
<td>6.8</td>
<td>–</td>
<td>PEI&lt;sup&gt;+&lt;/sup&gt;</td>
<td>–</td>
<td>1200 – 60</td>
<td>For bilayer 182</td>
</tr>
<tr>
<td>Concanavalin</td>
<td>104000</td>
<td>5</td>
<td>7</td>
<td>–</td>
<td>PEI&lt;sup&gt;+&lt;/sup&gt;</td>
<td>20</td>
<td>360 ± 50</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>186000</td>
<td>4.2</td>
<td>6.5</td>
<td>–</td>
<td>PEI&lt;sup&gt;+&lt;/sup&gt;</td>
<td>20</td>
<td>2150 ± 50</td>
<td>344 ± 8</td>
</tr>
<tr>
<td>Catalase</td>
<td>240000</td>
<td>5.5</td>
<td>9.2</td>
<td>–</td>
<td>PEI&lt;sup&gt;+&lt;/sup&gt;</td>
<td>–</td>
<td>For bilayer 500</td>
<td>For bilayer 80</td>
</tr>
<tr>
<td>Invertase</td>
<td>270000</td>
<td>3.8</td>
<td>7.0</td>
<td>–</td>
<td>PDDA&lt;sup&gt;+&lt;/sup&gt;</td>
<td>–</td>
<td>300 ± 50</td>
<td>48 ± 8</td>
</tr>
<tr>
<td>Diaphorase</td>
<td>600000</td>
<td>5</td>
<td>8</td>
<td>–</td>
<td>PEI&lt;sup&gt;+&lt;/sup&gt;</td>
<td>–</td>
<td>For bilayer 1300</td>
<td>For bilayer 208</td>
</tr>
</tbody>
</table>
Table 2-6. The LbL assembly of proteins with various polyelectrolytes or colloids. Some proteins are shown not to adsorb electrostatically in bilayers with the polyelectrolyte. This table was obtained from [246].

<table>
<thead>
<tr>
<th>Protein</th>
<th>Assembly plus</th>
<th>Polyelectrolyte</th>
<th>Charge</th>
<th>pH</th>
<th>Molarity</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidase</td>
<td>186 000</td>
<td>+PDPA</td>
<td>6.8</td>
<td>4.2</td>
<td>95 000</td>
<td>4.2</td>
</tr>
<tr>
<td>Glucuronidase</td>
<td>4.2</td>
<td>6.8</td>
<td>95 000</td>
<td>4.2</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>69 000</td>
<td>+HVd</td>
<td>6.8</td>
<td>4.2</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17 800</td>
<td>7.0</td>
<td>4.2</td>
<td>11 000</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Lysosome</td>
<td>15 300</td>
<td>+HVa</td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Cytochrome</td>
<td>12 400</td>
<td>+PSA</td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein</th>
<th>Assembly plus</th>
<th>Polyelectrolyte</th>
<th>Charge</th>
<th>pH</th>
<th>Molarity</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidase</td>
<td>186 000</td>
<td>+PDPA</td>
<td>6.8</td>
<td>4.2</td>
<td>95 000</td>
<td>4.2</td>
</tr>
<tr>
<td>Glucuronidase</td>
<td>4.2</td>
<td>6.8</td>
<td>95 000</td>
<td>4.2</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>69 000</td>
<td>+HVd</td>
<td>6.8</td>
<td>4.2</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17 800</td>
<td>7.0</td>
<td>4.2</td>
<td>11 000</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Lysosome</td>
<td>15 300</td>
<td>+HVa</td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Cytochrome</td>
<td>12 400</td>
<td>+PSA</td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

Note: The table was obtained from [246].
Enzymes are a specific class of proteins that possess charged functional groups on their surfaces while solubilized in an aqueous medium, hence they can be immobilized within LbL films through their electrostatic interactions with other polyelectrolytes [249]. Enzymatic activity has been shown to increase linearly with every layer of enzyme that is deposited, even on a rough porous membrane surface; however, the enzymatic activity reaches a plateau after a certain number of polyelectrolyte layers has been deposited because of an increased substrate diffusion resistance [237].

The polyelectrolyte films fabricated by LbL techniques are ultrathin (much less than 1 μm in thickness) [232, 246]. Therefore, the quantity of adsorbed polyelectrolytes is small and is usually of the order of $10^{-9} – 10^{-7}$ g cm$^{-2}$ [237, 256]. This can be important when expensive enzymes, such as CA, are used.

### 2.12 Concluding Remarks

Alkanolamines such as MEA and DEA have been well characterized and investigated for CO$_2$ capture from flue gases [1, 17, 18, 30], even though these solvents consume much energy during the desorption process. The use of other solvents such as K$_2$CO$_3$ or tertiary alkanolamines requires less energy for the desorption of CO$_2$, but the absorption kinetics are still noticeably much slower than MEA or DEA. They thus require the use of additional promoters to increase the reaction rate for CO$_2$ absorption.

The use of CA to facilitate the absorption of CO$_2$ within such solvents shows promise. However, the large scale incorporation of CA into existing CO$_2$ absorption processes, which operate at both elevated temperature and ionic strength has not been well documented, particularly over long time frames. The stability of CA under such conditions is critical to the economic feasibility of the approach.

This literature review has shown that if CA is to be used, it needs to be immobilized in the absorber, as it is highly unlikely to survive at
the temperatures in the stripper. In addition, the enzyme must be located close to the gas-liquid interface, as otherwise the liquid phase resistance to mass transfer becomes rate controlling, so that the CA is rendered ineffective [227].

The use of membrane contactors for CO$_2$ absorption processes has been investigated significantly. Much light has been shed on the high surface area-volume ratio of hollow fiber membranes, which dramatically reduces the footprint and the operating costs of the absorption process in comparison to an absorption column. While PTFE may seem to be the best material for fabricating membrane contactors, PP is still the most widely used material for fabricating hollow fiber membrane contactors at the moment because of its relatively lower cost and ready availability in comparison with PP. However, PP membrane contactors are incompatible with MEA and are readily wetted by MEA, which translates into an increased mass transfer resistance and a reduced overall mass transfer coefficient.

Multiple methods to reduce pore wetting have been proposed, such as the use of alternative solvents that have higher surface tensions, are less volatile and less environmentally unfriendly than MEA. However, the biggest issue with these solvents is that they exhibit much lower rates of CO$_2$ absorption in comparison with MEA.

An alternative method to reduce the pore wetting is to coat a thin dense layer on top of the membrane to reduce the pore size, but mass transfer rates are also affected by the presence of that dense layer unless the thickness of the dense layer is extremely small. To simultaneously boost the CO$_2$ reaction rates and reduce the effects of pore wetting, CA films can be adsorbed electrostatically onto membrane surfaces via LbL assembly in ultrathin films. This would allow the CA to be located extremely near the gas-liquid interface, which would facilitate rapid CO$_2$ absorption. These films can then be investigated for the CO$_2$ hydration activity and their capabilities in enhancing the CO$_2$ mass transfer rates when immobilized onto membrane contactors.
A better characterization of a long-term CA-facilitated CO$_2$ MGA process coupled with a thorough understanding of the process economics behind the use of CA would be tremendously beneficial to reducing the overall energy consumption of the current absorption processes.

2.13 Scope of Thesis

This thesis investigates the fabrication of ultrathin films containing CA onto membrane surfaces for enhancing the absorption rate of CO$_2$ into a K$_2$CO$_3$ solvent.

Chapter 3 covers the materials and methods that are used for adsorbing the enzyme on both flat sheet membrane coupons. Analytical techniques for characterizing the enzyme activity and the rate of CO$_2$ absorption are outlined. Methods for qualitative characterization of the membranes after LbL assembly are also outlined.

Chapter 4 provides an insight into the fabrication of ultrathin CA films on the surface of planar PP membranes. It is shown in this chapter that the immobilized enzymes are able to retain their activity and increase the rate of CO$_2$ hydration. The addition of mesoporous silica nanoparticles further increases the enzyme loading and the overall enzyme activity; however, the specific enzyme activity is decreased after immobilization onto those nanoparticles.

In Chapter 5, the LbL assembly technique is optimized for application on hollow fiber PP (microporous) and PDMS (nonporous) membranes. Electrostatic adsorption is conducted by pumping the polyelectrolyte solutions through hollow fiber modules to coat the shell side of the hollow fibers with polyelectrolyte and protein. The pumping flow rates and the adsorption time used per layer of polyelectrolyte are investigated. The effects of temperature on CO$_2$ absorption are also considered. The mesoporous silica nanoparticles were not considered for LbL film assembly because of the severe reductions observed in the specific enzyme activity as shown in Chapter 4, as well as the fact that
silica nanoparticles are known to degrade under highly alkaline conditions. A model of the mass transfer process is developed based on suitable correlations that are outlined in the literature review above.

Chapter 6 focuses on the long-term operation of the nonporous PDMS membrane contactors to further understand the enzyme deactivation kinetics across a range of temperatures. The enzyme is also contacted with toxic gases such as SO$_2$ and NO to understand the effects of these gases on the enzyme for the purpose of simulating industrial conditions.

Finally, Chapter 7 provides a series of concluding remarks on how future work can be conducted to build on the groundwork that has been laid by this thesis.
Chapter 3: Materials and Methods

3.1 Materials Used

All chemicals were used as purchased unless otherwise stated. PEI (25 kDa), PSS (70 kDa), PAH (10 kDa), para-nitrophenol (p-NP), trishydroxymethylaminomethane (Tris), para-nitrophenylacetate (p-NPA) and were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Concentrated hydrochloric acid (HCl, 37%) and concentrated sulfuric acid (H₂SO₄, 98%) were purchased from Scharlau (Gillman, SA, Australia). Compressed CO₂ was supplied by Coregas (Thomastown, VIC, Australia). Calcium chloride dihydrate (CaCl₂·2H₂O), sodium chloride (NaCl), potassium chloride (KCl), sodium hydroxide (NaOH) and 30% hydrogen peroxide (H₂O₂) were obtained from ChemSupply (Gillman, SA, Australia). K₂CO₃ (≥ 99%) was obtained from Thasco Chemical Co. Ltd (Bangkok, Thailand) and dissolved in tap water to prepare a 30 wt% K₂CO₃ solution. The physical properties of this solvent are provided in Table 3-1.

<table>
<thead>
<tr>
<th>Table 3-1. Properties of 30 wt% K₂CO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solvent Parameters</strong></td>
</tr>
<tr>
<td>Density (kg m⁻³) [82]</td>
</tr>
<tr>
<td>CO₂ Diffusivity (m² s⁻¹) [109]</td>
</tr>
<tr>
<td>Viscosity (mPa s) [109]</td>
</tr>
</tbody>
</table>

A suspension of silica particles (SP, 50 mg mL⁻¹, 2.78 μm diameter) in water was obtained from Microparticles GmbH (Berlin, Germany).

Ultrapure water was obtained from a Milli-Q purification system (Merck Millipore, Kilsyth, VIC, Australia) with a resistivity of more than
18.2 MΩ cm. Purified water was obtained from a Millipore purification system (Merck Millipore, Kilsyth, VIC, Australia) with a resistivity of > 5.0 MΩ cm. Tris buffer (10 mM or 50 mM, pH 6.4 or pH 7.2) was prepared in ultrapure water and the pH was adjusted by dropwise addition of hydrochloric acid (1 M). The polyelectrolytes (i.e., PEI, PSS, and PAH) solutions were all prepared in Tris buffer (pH 7.2) at a concentration of 1 mg mL⁻¹.

Two sources of CA were used. The first source was a commercial form derived from bovine erythrocytes, referred to hereafter as BCA (Sigma product C-3934) and provided in a powder form, with an activity of 2500 Wilbur-Anderson units per mg of protein. BCA with a concentration of 0.2 mg mL⁻¹ was prepared in Tris buffer (pH 7.2) prior to use.

The second source was an experimental thermostable microbial CA produced by Novozymes A/S (Bagsvaerd, Denmark), referred to hereafter as NCA. The enzyme was produced by microbial fermentation in a benign host organism, which was removed during recovery of the enzyme broth and is not present in the sample. The fermentation broth was stored at -4 °C and only thawed to room temperature when it was required for use. This broth was diluted to 2% (v/v) with Tris buffer (pH 7.2) prior to LbL adsorption and will be denoted as NCA. The 2% NCA solution was filtered through 0.22 μm syringe filters to remove suspended solids for zeta potential and quartz crystal microgravimetry (QCM) analysis.

Mesoporous silica (MS) nanoparticles with an average size of 110 nm were synthesized according to a previously reported method [259]. These particles were suspended in Tris buffer (pH 7.2) containing 0.1 M NaCl [250] to a particle concentration of 5 mg mL⁻¹ and sonicated for 5 min to ensure an even dispersion of the particles prior to use.

Flat sheet PP membrane coupons were obtained from Sterlitech Corporation (Kent, WA, USA). These membranes have a pore size of 0.10
μm, a thickness of 75-110 μm and a diameter of 47 mm, according to information provided by the supplier.

Porous PP hollow fiber membranes were obtained from Memtec Australia. Polysulfone (PS) hollow fiber membranes that were coated with a 0.5 μm layer of non-porous polydimethoxysilane (PDMS) were supplied by Airrane Co. Ltd. (Daejeon, Korea). The parameters for these hollow fibers are shown in Table 3-2.

Table 3-2. Membrane module operating parameters

<table>
<thead>
<tr>
<th>Membrane Parameter</th>
<th>PP</th>
<th>PDMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore Diameter (μm)</td>
<td>0.2</td>
<td>Nonporous</td>
</tr>
<tr>
<td>Fiber Internal Diameter $d_{in}$ (mm)</td>
<td>0.24$^a$</td>
<td>0.30$^a$</td>
</tr>
<tr>
<td>Fiber External Diameter $d_{out}$ (mm)</td>
<td>0.52$^a$</td>
<td>0.45$^a$</td>
</tr>
<tr>
<td>Module Hydraulic Diameter $d_H$ (mm)</td>
<td>0.59</td>
<td>0.81</td>
</tr>
<tr>
<td>Porosity</td>
<td>0.65</td>
<td>Nonporous</td>
</tr>
<tr>
<td>Tortuosity</td>
<td>2.5</td>
<td>Nonporous</td>
</tr>
<tr>
<td>Packing Density</td>
<td>0.40</td>
<td>0.30</td>
</tr>
</tbody>
</table>

$^a$These values were obtained from cross-sectional scanning electron microscopy (SEM) images.

The hollow fiber membranes were packed into a membrane module housing with an effective length of 20 cm in bundles of 20 with a packing density of 65%. The ends of the module housing were sealed with an epoxy adhesive (Selleys Araldite Super Strength Epoxy Adhesive, Selleys Pty Ltd, NSW, Australia) before conducting any experiments on the membranes.

3.2 Enzyme Immobilization

3.2.1 Enzyme Immobilization onto Smooth Non-Porous Surfaces

Glass slides (22 mm × 22 mm) or silicon wafers were cleaned with piranha solution (30% H$_2$O$_2$ mixed with 70% H$_2$SO$_4$. Caution! Piranha solution is extremely reactive and corrosive, only small volumes should be prepared freshly for use) for 5 minutes, washed with ultrapure water.
and then subsequently with ethanol, dried under a stream of nitrogen gas and irradiated with ultraviolet rays for 20 minutes.

A PEI/[PSS/PAH]$_2$ precursor film was first deposited on the glass slide or silicon wafer by immersion into the respective polyelectrolyte solutions for 5 min. After the deposition of each layer, excess polyelectrolyte was removed by rinsing with excess Tris buffer (pH 7.2) for 3 min.

BCA or NCA was then adsorbed sequentially with the polyelectrolytes to form multilayered enzyme films. Both sides of the glass slides were exposed to the solutions.

3.2.2 Enzyme Immobilization onto Flat Sheet Membranes

PP membrane coupons were placed onto a glass substrate. Polyelectrolyte solutions were coated onto the active side of the membrane surface using a commercial paintbrush (Woolworths Ltd, Bella Vista, New South Wales, Australia) to spread the solution evenly across the membrane. Each polyelectrolyte solution was left on the surface of the membranes for 3 min before rinsing with excess Tris buffer (pH 7.2).

3.2.3 Enzyme Immobilization with MS Nanoparticles

To investigate the effect of MS nanoparticles on the adsorption and activity of immobilized NCA, fresh glass slides or membrane coupons were coated sequentially as [MS/NCA/PAH]$_2$/MS/NCA after the deposition of the precursor film. The membranes were contacted with the MS particles or the NCA solution for 20 min before washing with buffer and then contacted with PAH for 5 min before washing with buffer. The film that was formed will be referred to as the MSNCA film throughout the rest of the thesis. The quantity of enzyme that was adsorbed was determined by analyzing the supernatant using UV-Vis spectrophotometry on a Varian Cary 1E UV-Vis spectrophotometer (Agilent Technologies, Mulgrave, VIC, Australia) with 10 mm quartz
cuvettes (Starna Pty Ltd, Baulkham Hills, NSW, Australia) at a wavelength of 280 nm.

3.3 Zeta-Potential (ζ) Measurements of the Silica Particles

To determine the zeta-potential of each polyelectrolyte layer, the polyelectrolytes were adsorbed onto commercial SPs. Approximately 1 mL of polyelectrolyte solution in Tris buffer (10 mM) was mixed with 100 μL of the SP suspension (50 mg mL⁻¹) for 5 min to deposit a polyelectrolyte layer. The particles were then spun down at 4000 g for 45 sec and the supernatant was discarded. The particles were redispersed in 700 μL of Tris buffer (10 mM, pH 7.2) via vortex and then centrifuged at 4000 g for 45 sec to remove free polyelectrolytes. The washing step was repeated three times to remove all free polyelectrolyte before the deposition of the next polyelectrolyte layer. Approximately 1 μL of the particle suspension was removed after the addition of each polyelectrolyte layer and mixed with 700 μL of Tris buffer (10 mM, pH 7.2) for zeta potential measurements on a Malvern Zetasizer Nano ZS instrument (Worcestershire, UK). A precursor PEI/[PSS/PAH]² film was deposited prior to introducing the CA enzyme, which was then either alternately layered with PAH or PSS onto the precursor film.

3.4 Quartz Crystal Microgravimetry

Quartz crystal microgravimetry (QCM) is an extremely useful and important technique in the field of LbL technology that analyzes thin films by quantifying the mass of any polyelectrolyte that is adsorbed onto a film layer [232, 240, 248, 258, 260]. The resonant frequency of a quartz crystal is changed upon the adsorption of polyelectrolytes onto the crystal, and the change in frequency (ΔF) is related to the change in mass (Δm_A) according to the Sauerbrey equation (Equation 3-1) [261]:

\[ ΔF = \frac{C \cdot Δm_A}{d} \]
\[
\Delta F = -2 \frac{f_0^2}{A \sqrt{\rho_q \mu_q}} \Delta m_A
\]  
(3-1)

where \(f_0\) is the resonant frequency of the quartz crystal, \(A\) is the active area of the crystal, \(\rho_q\) is the density of quartz and \(\mu_q\) is the shear modulus of quartz. The thickness of the film layers can also be estimated from the Sauerbrey equation by deconstructing \(\Delta m_A\) into its density and volume components [232, 246].

However, Equation 3-1 is valid only if the film is rigid. The rigidity of the deposited film is defined by the value of the dissipation factor \(D_i\) relative to the crystal oscillation frequency \(F\), which is given in Equation 3-2 [260]:

\[
D_i = \frac{1}{\pi t_D F_{t_D}}
\]  
(3-2)

where \(t_D\) is the decay time, which is the time taken for the oscillation wave to decay upon the deposition of mass onto the crystal.

A film is said to be rigid when the oscillation wave decays rapidly, such that the change in the dissipation factor \(\Delta D_i\) is less than \(10^{-7}\) times the change in the frequency \(\Delta F\) when a polyelectrolyte layer is deposited onto the crystal [262]. Should the film be not rigidly adhered to the electrode surface or is not evenly deposited, Equation 3-3 is applicable for quantifying film growth [263]:

\[
\Delta F = -f_0^{1.5} \frac{\sqrt{\rho_L \eta_L}}{\sqrt{\pi \rho_q \mu_q}}
\]  
(3-3)

where \(\rho_L\) is the liquid density and \(\eta_L\) is the liquid viscosity, with all other terms being defined as per Equation 3-1.

QCM was used to determine the mass of enzyme adsorbed per unit area on AT cut 5 MHz QCM quartz crystals using a Q-Sense E4 Auto system to quantify the changes in the resonant frequency. Prior to QCM experiments, the crystal surfaces were cleaned with piranha solution (30% H₂O₂ mixed with 70% H₂SO₄. Caution! Piranha solution is extremely reactive and corrosive, only small volumes should be prepared freshly for use) for 5 minutes, washed with Milli-Q water and then
subsequently with ethanol, dried under a stream of nitrogen gas and irradiated with ultraviolet rays for 20 minutes. The changes in the resonance frequency of the crystals during multilayer deposition were monitored at the third overtone at room temperature.

Deposition steps and washing steps were conducted for durations of 3 minutes and 2 minutes, respectively, with 250 μL aliquots of polyelectrolyte solution or Milli-Q water at a flow rate of 0.579 mL min⁻¹. After the formation of a PEI/[PSS/PAH]₂ precursor film for regular stepwise film growth [232], CA was deposited onto the quartz crystals sequentially with the polyelectrolytes to form multilayer enzyme films. QCM was also used to investigate the construction of NCA films in a similar manner.

Equation 3-1 was simplified down to Equation 3-4 and used to determine the mass of material deposited onto the quartz crystal, which had an active area of 1 cm in diameter. The change in the resonant frequency (ΔF) of the quartz crystals is linearly dependent on the mass of material (Δmₐ) deposited onto the crystal:

\[
\Delta F = - \frac{n_o \Delta m_A}{C}
\]  

(3-4)

where \( n_o \) is the number of overtones and \( C \) is a constant (17.7 ng Hz⁻¹ cm⁻² for a 5 MHz AT cut crystal from Q-Sense). Equation 3-4 can be rewritten to relate the layer thickness (\( x \)) to the change in the observed resonant frequency of the crystal (ΔF) and the density of the film (\( \rho_f \)) [232]:

\[
x = -\left(2.12 \times 10^{-4}\right) \frac{\Delta F}{\rho_f}
\]

(3-5)

The surface area of the crystal can be estimated to be approximately 20% larger after enzyme deposition because of an increased surface roughness created by the adsorption of the polyelectrolytes.[246] The densities of generic protein and polyelectrolyte layers have been previously assumed to be approximately 1.3 ± 0.1 g cm⁻³ and 1.2 ± 0.1 g cm⁻³, respectively [246]. For estimating the
maximum theoretical film thickness of the hybrid polyelectrolyte/protein film, a film density of 1.2 ± 0.1 g cm\(^{-3}\) was assumed in this case.

### 3.5 Electron Microscopy

The MS nanoparticles were imaged by transmission electron microscopy (TEM) using a FEI Tecnai TF20 microscope at a voltage of 200 kV after being placed onto Formvar-coated copper grids and dried in air.

The glass slides and the membrane coupons containing adsorbed enzyme were dried under a nitrogen stream, gold sputter-coated and analyzed using a Phillips XL30 FEG field emission scanning electron microscope (FESEM) at a voltage of 2.0 kV to determine the surface morphologies of the glass slides and the membrane coupons before and after protein adsorption.

Wetted membrane coupons were immersed in liquid nitrogen for 5 min and then fractured for an analysis of the membrane cross-section.

### 3.6 Characterization of the Flat Sheet Membranes

The liquid breakthrough pressure (LBP) of the membranes was measured by placing a membrane coupon of 4.8 cm in diameter within a dead-end filtration cell (Sterlitech HP4750, Kent, WA, USA) that had an active filtration area of 3.2 cm in diameter. The cell was pressurized with air and increased in 50 kPa steps every 5 min to determine the pressure that was sufficient to force water through the membrane. Water flux tests were conducted at a constant pressure (greater than the LBP) to determine the permeability of the membrane. The membranes were then air dried after the water flux tests for 72 h. Contact angle measurements were performed on the dry membranes using a FTÅ200 dynamic contact angle analyzer (First Ten Ångstroms, Portsmouth, VA, USA).
3.7 Characterization of Enzyme Activity

The enzymatic activity of the adsorbed enzyme on the glass slides was initially determined based on the rate that the adsorbed BCA converted \( p\)-NPA to \( p\)-NP. Solutions of \( p\)-NPA dissolved in acetonitrile (2.5 mg mL\(^{-1}\)) were mixed with Tris buffer (pH 7.2) at a volumetric ratio of 1:9 [144, 156]. CA-coated glass slides were incubated within a 20 mL aliquot of this \( p\)-NPA solution, and a small volume of the mixture was extracted at set time points and analyzed by UV-Vis spectrophotometry at 348 nm to determine the total concentration of \( p\)-NP and \( p\)-nitrophenoxide in solution [163] for calculating the rate of \( p\)-NPA hydrolysis.

The activity of the immobilized enzymes on the glass slides and the membranes were also quantified for their ability to hydrate CO\(_2\) into HCO\(_3^-\); based on the method provided by Vinoba and co-workers [48, 49]. A saturated CO\(_2\) solution was prepared by passing a CO\(_2\) gas stream into a bottle of ultrapure water at 25°C for 1 hour. Approximately 40 mL of Tris buffer (pH 6.4) was added to 100 mL of the saturated CO\(_2\) solution before the enzyme-coated glass slide was immersed into the mixture. The mixture was stirred for 1 minute followed by the removal of the glass slide or the membrane coupon before the addition of 20 mL of CaCl\(_2\)·2H\(_2\)O solution (5.3% w/v) and 10 mL of NaOH solution (1 M) to quench the reaction and precipitate the Ca(HCO\(_3\))\(_2\) as CaCO\(_3\). The precipitated CaCO\(_3\) was then filtered, dried in an oven at 80°C overnight and weighed to determine the quantity of CO\(_2\) that had been hydrated based on the quantity of CaCO\(_3\) produced. This activity assay may not be the most optimum for determining CA activity as the presence of the acetonitrile solvent can affect enzyme performance and as the low pH (6.4) reduces the concentration of hydroxyl ions available for reaction. However, this method was consistently used throughout the thesis, so relative changes in the CA activity will be accurately recorded.
A separate activity test was used for the determination of the free NCA activity in solution. A sample of 2% NCA in Tris buffer (pH 7.2) was sequentially diluted to various levels of dilution, and 1 mL aliquots of the diluted enzyme was added to different batches of 100 mL saturated CO₂ solution. The mixture was stirred for 1 minute before adding 20 mL of CaCl₂·2H₂O solution (5.3% w/v) and 10 mL of NaOH solution (1 M) to quench the reaction. The precipitated CaCO₃ was then filtered and dried before determining the quantity of CaCO₃ produced.

Control experiments were conducted to determine the quantity of CaCO₃ that was produced in the absence of any enzyme. Any incremental CaCO₃ that was produced in the presence of CA was then attributed to the hydration activity of the enzyme.

Free NCA samples and membrane samples containing adsorbed NCA were also directly compared for their pH stability via incubation either in a 0.2 M KCl solution (adjusted to pH 12 with NaOH) or in Tris buffer (pH 7.2) for 72 h at 4°C. The activity test was then repeated after the incubation step.

3.8 Layer-by-Layer Electrostatic Adsorption on Hollow Fiber Membrane Contactors

3.8.1 Dynamic LbL Adsorption

Polyelectrolyte solutions were pumped through PP hollow fiber membrane modules using a peristaltic pump so that they contacted with the shell side of the hollow fibers. The solutions were continuously circulated through the membrane module, followed by a washing of the hollow fibers with ultrapure water for 10 minutes before the next polyelectrolyte solution was pumped through the module at the same flow rate. A film was fabricated onto the hollow fibers from the adsorbed polyelectrolytes. This film was prepared in the form of a PEI/PSS/PAH/[PSS/PAH/NCA]ₓ film, where x refers to the number of cycles of [PSS/PAH/NCA] trilayers deposited for film fabrication. The flow rate of the polyelectrolyte solutions and the contact time with the
membrane were varied for optimization purposes. Two membrane modules were prepared for each condition to allow the reproducibility to be determined. Other PP membrane modules were also prepared with adsorbed PEI/[PSS/PAH]₄ polyelectrolyte films in a similar manner but without any NCA as a control system.

3.8.2 Static LbL Adsorption

PP and PDMS hollow fiber modules were prepared by pumping polyelectrolyte solution into the module chamber until the chamber was filled with polyelectrolyte solution. The solution was then allowed to remain in contact with the hollow fibers for 5 min in a static mode before washing with ultrapure water at 20 mL/min for 10 min. The module was then cleared of water before the next polyelectrolyte solution was pumped into the module for the fabrication of subsequent layers. Some modules were dried by passing nitrogen gas through the lumen side of the hollow fibers at a flow rate of 1 L/min for 2 h, while others were used directly for contactor experiments.

3.8.3 Permeation Mode

Other PP modules were also prepared by forcing polyelectrolyte solution across the membrane into the lumen side. This was done by using a needle valve to restrict the flow of the polyelectrolyte solution out of the shell side exit, which increased the transmembrane pressure to a point where the solution could penetrate the membrane structure and cause complete wetting. The hollow fibers were then dried by passing nitrogen gas through the lumen side of the hollow fibers at a flow rate of 1 L/min for 2 h.

3.9 Operation of Hollow Fiber Membrane Contactors

A pilot plant gas absorption unit described previously [6] was utilized for these experiments. Pure gaseous CO₂ (99%, Coregas, Thomastown, VIC, Australia) at 1.5-2.0 bar g was used as feed to the
lumen side of the hollow fibers, with the feed gas flowrate measured by a gas rotameter from Kytola (Muurame, Finland) and the retentate flowrate measured by a gas meter (Ampy Email metering Model 750). The 30 wt% potassium carbonate solvent was pumped through the shell side of the fibers (Micropump with an Ismatec controller) at a pressure differential of 0.1 bar above the gas pressure to prevent bubble formation within the solvent for the microporous membranes. The flowrate was measured by another Kytola rotameter and controlled by the pump speed. The solvent flowrate was maintained for 1 h to ensure that steady state was reached before measurements were taken.

Figure 3-1. A schematic diagram showing the process for the MGA operation. FIC = flow indicator and control, FI = flow indicator, TI = temperature indicator, PI = pressure indicator and PIC = pressure indicator and control. This figure was obtained from [6].

The solvent flowrate was maintained for 1 h to ensure that steady state was reached before it was changed. All modules were contacted with 30 wt% K$_2$CO$_3$.

3.9.2 Solvent Desorption and Reuse

For long term operations, the spent K$_2$CO$_3$ solvent was circulated on the shell side of a second PDMS hollow fiber module containing 20 hollow fiber membranes of the same type and length as the absorption membrane unit. Pure nitrogen gas (1 L/min) was used as a sweep gas through the lumen of the hollow fibers to remove excess solubilized CO$_2$ from the K$_2$CO$_3$ solvent that was pumped through the shell side of the
fibers. The temperature of the stripping operation was regulated between 70-100°C and the solvent flow rate was controlled at 0.01-0.03 L min⁻¹ to maintain a consistent K₂CO₃ solvent concentration for recirculation into the absorption unit at a KHCO₃ loading of 0.1-0.15, while the solvent flow rate was kept constant at 0.05 L min⁻¹. Absorption was conducted over prolonged periods of time (100-600 h) at different temperatures.

3.10 Analysis of Bicarbonate and Carbonate Concentrations in the Spent Absorption Solvent

Samples of spent solvent exiting the contactor were collected and then analyzed for their K₂CO₃ and KHCO₃ concentrations via titration with a Metrohm–Titrando 809 (Switzerland) auto-titrator that was coupled with a high temperature Metrohm (Switzerland) pH probe. Approximately 5 mL of solvent was diluted with 60 mL of purified water using a set volume liquid dispenser (Eppendorf, Varispencer, Germany). This mixture was then titrated against a 0.4 M sulfuric acid solution. The titration data were collected via a computer using the Metrohm tiamo software package [103], which determined the HCO₃⁻ and CO₃²⁻ concentrations within the solvent based on the volumes of sulfuric acid that were required to reach the HCO₃⁻ and the H₂CO₃ equivalence points. The outlet bicarbonate concentrations were then used to determine the CO₂ flux through the hollow fiber membrane, which is based on the CO₂-K₂CO₃ reaction stoichiometry that was presented in Equation 2-2.

3.11 Exposure of Immobilized CA to Toxic Gases

Flat sheet PP membrane coupons were coated with NCA based on Section 3.4.2 to form a PEI/PSS/PAH/[PSS/PAH/NCA]₂ film. Moist PP membrane coupons with adsorbed enzyme were placed in a stainless steel vessel. Other membrane coupons were dried under a stream of N₂ gas for 3 min before being placed in the vessel. The vessel was purged
with N\textsubscript{2} at atmospheric pressure (1 bar abs) and pressurized with 1000 ppm of SO\textsubscript{2} or NO in N\textsubscript{2} to 2 bar abs for a final SO\textsubscript{2} or NO concentration of 500 ppm in N\textsubscript{2}, resulting in a final gas partial pressure of 1 bar abs. The membrane coupons were removed from the vessel periodically to determine the enzyme activity. After each removal, the vessel was purged with N\textsubscript{2} and re-pressurized again with 500 ppm of SO\textsubscript{2} or NO in N\textsubscript{2}.
Chapter 4: Surface Engineering of Polypropylene Membranes with Carbonic Anhydrase-Loaded Mesoporous Silica Nanoparticles for Improved Carbon Dioxide Hydration

4.1 Chapter Perspective

This chapter focuses on the fabrication of thin films containing carbonic anhydrase (CA) onto flat planar surfaces. The layer-by-layer (LbL) technique is used for film fabrication, where polyelectrolytes can be adsorbed onto a flat nonporous surface in uniform layers. Quartz crystal microgravimetry (QCM) is used to quantify the mass of CA that is adsorbed onto a nonporous quartz crystal surface, while an enzyme activity assay was be developed for quantifying the activity of the adsorbed CA to correlate the mass of CA that is adsorbed onto the nonporous quartz crystal with the mass of CA that is adsorbed onto the porous membrane surface. Mesoporous silica (MS) nanoparticles are added to the film to increase the enzyme loading. It was determined that the MS nanoparticles were able to increase the enzyme loading, though the specific enzyme activity was found to decrease significantly, which could be attributed to an increased diffusional resistance for the substrate to reach the enzyme molecules that were adsorbed within the nanoparticles.

4.2 Abstract

Carbonic anhydrase (CA) is a native enzyme that facilitates the hydration of carbon dioxide into bicarbonate ions. This study reports the fabrication of thin films of active CA enzyme onto a porous membrane substrate using the layer-by-layer (LbL) assembly. Deposition of multilayer films consisting of polyelectrolytes and CA was monitored by quartz crystal microgravimetry, while the enzymatic activity was assayed according to the rates of $p$-nitrophenylacetate ($p$-NPA) hydrolysis and CO$_2$ hydration. The fabrication of the films onto a nonporous glass substrate showed CO$_2$ hydration rates of $0.52 \pm 0.09 \mu\text{mol \ cm}^{-2} \ \text{min}^{-1}$ per layer of bovine CA and $2.6 \pm 0.7 \mu\text{mol \ cm}^{-2} \ \text{min}^{-1}$ per layer of a thermostable microbial CA. The fabrication of a multilayer film containing the microbial CA on a porous polypropylene membrane increased the hydration rate to $5.3 \pm 0.8 \mu\text{mol \ cm}^{-2} \ \text{min}^{-1}$ per layer of microbial CA. The addition of mesoporous silica nanoparticles as a film layer prior to enzyme adsorption was found to increase the activity on the polypropylene membranes even further to a rate of $19 \pm 4 \mu\text{mol \ cm}^{-2} \ \text{min}^{-1}$ per layer of microbial CA. The LbL treatment of these membranes increased the mass transfer resistance of the membrane but decreased the likelihood of membrane pore wetting. These results have potential application in the absorption of carbon dioxide from combustion flue gases into aqueous solvents using gas-liquid membrane contactors.
4.3 Introduction

Carbon dioxide (CO$_2$) emissions into the atmosphere are of great concern because of the environmental impact associated with climate change. The increase in CO$_2$ emissions is mainly derived from human activity and has followed an increasing trend over the past few decades [2, 17]. Hence, it is important to curb the rate of CO$_2$ emissions into the atmosphere through technologies such as carbon capture and sequestration operations.

The hydration of CO$_2$ into HCO$_3^-$ is known to be the rate-limiting step when absorbing carbon dioxide into an aqueous medium [264]. Hydration rates can be accelerated by a naturally occurring enzyme known as carbonic anhydrase (CA) [147]. Therefore, efforts have been made to adsorb CA onto surfaces such as polymethylpentene hollow fiber membranes [51], hydrogels [154], activated carbon particles [35], mesoporous silica [48], gold nanoparticles conjugated with silica [49] and polyurethane films [144], such that they can be used as immobilized solid-state catalysts in CO$_2$ capture operations.

Polymeric membranes are promising substitutes for absorbing gaseous CO$_2$ into an aqueous solution. In membrane-based CO$_2$ absorption operations, a feed gas containing CO$_2$ is pumped through one side of the membrane, while the other side of the membrane contains the solvent for absorbing the CO$_2$ [92]. The membrane is hydrophobic to prevent solvent leakage into the gas stream. Such membrane contactors are readily scalable [92] and are known to produce CO$_2$ streams of high purity even at high CO$_2$ removal rates [9]. The immobilization of CA onto these membranes would further enhance the rate of CO$_2$ absorption into the solvent. However, the CA layer that is immobilized onto the membrane will also add to the mass transfer resistance of CO$_2$ from the gas phase to the gas-liquid interface. It is preferable that the CA film layer be as thin as possible to reduce the mass transfer resistance [265], which also allows the gas-liquid
interface to be closer to the enzyme molecules for better reaction rates [226].

Layer-by-layer (LbL) assembly is a well-established technique for the preparation of thin multilayered films of oppositely charged polyelectrolytes onto solid planar substrates [230] or particles [250]. The adsorption of proteins, such as immunoglobulin G [240], glucose oxidase [234] and peroxidase [237] using LbL assembly, have been investigated with the aim of developing new immunosensors [240] or immobilized catalysts [246]. The construction of CA bilayers with polyethylene imine (PEI) by the LbL technique has also been reported, with a CA loading of 280 ng cm\(^{-2}\), as determined by quartz crystal microgravimetry (QCM) [220].

It has been shown that the immobilization of CA onto nanoparticles may further enhance the enzyme activity [49], as there is a greater degree of freedom for the enzyme to attach to the curved surfaces of the nanoparticles [221] and hence preserve the active sites [222]. For example, nonporous silica nanoparticles containing adsorbed CA have already been examined for their ability to hydrate CO\(_2\) [55]. Mesoporous nanoparticles can further increase the enzymatic loading in comparison with nonporous nanoparticles by providing high surface areas with pores that are similar to the size of the target enzyme molecules [251, 266]. Nanosized particles are selected in preference to micron sized particles to reduce the need for intra-particular mass transfer of CO\(_2\), as such transport limitations have been shown to cause a reduction in the overall kinetics [227].

It has been shown that such nanoparticles can be dispersed within a polymer solution prior to casting as a flat sheet membrane, but that these nanoparticles are prone to leaching [222]. Alternatively, submicrometer hollow spheres that are resistant to leaching from aqueous solvents have been fabricated through the LbL technique using alternating layers of silica nanoparticles and polyelectrolytes [250]. Enzymes that are immobilized within such LbL films are known to
exhibit enhanced stabilities relative to their free states and can maintain their activity levels within the film for a longer period of time [53].

In this chapter, we report the fabrication of CA multilayer films both with and without the addition of mesoporous nanoparticles. We investigate the activity of the resulting films when deposited on both a smooth nonporous planar glass substrate and a commercial hydrophobic polypropylene porous membrane. The CA that was adsorbed within the films was found to increase the rate of CO$_2$ conversion into HCO$_3^-$ ions, with a linear correlation between the number of CA layers adsorbed and the quantity of CO$_2$ converted into HCO$_3^-$. The specific enzyme activity was found to increase relative to the free enzyme when it was immobilized within a polyelectrolyte film, but there was a significant decrease in the specific activity upon immobilization within the mesoporous nanoparticles. In addition, the films also provided increased resistance against water penetration into the membrane pores even though they caused the membrane surface to become more hydrophilic.

4.4 Enzyme Adsorption and Multilayer Film Formation

Zeta-potential measurements of PAH/CA and PSS/CA layers deposited onto silica spheres at pH 7.2 confirmed that both BCA and NCA are negatively charged (Figure 4-1).
Figure 4-1. Zeta-potential measurements for PAH/BCA, PSS/BCA, PAH/NCA and PSS/NCA bilayers prepared in a Tris buffer solution at pH 7.2. The odd numbered layers (1, 3 and 5) represent PAH or PSS layers, while the even numbered layers (2, 4 and 6) represent BCA or NCA layers.

However, the inability of either enzyme to reverse the surface zeta potential when applied after the positively charged PAH layer indicates that these proteins possess only a weak net negative charge at the measurement pH of 7.2. This weak net negative charge is consistent with the reported isoelectric points for commercial BCA, which range from pH 4.9 to pH 6.7 [267].

The lack of surface charge inversion prevents the uniform addition of extra PSS/CA or PAH/CA bilayers sequentially beyond the first bilayer, as it is known that the way to develop stepwise growth in multilayer film formation is to achieve a complete reversal in the surface charge [232]. This is demonstrated by the limiting changes in zeta-potential for successive PSS/CA and PAH/CA bilayers in Figure 4-1.
Consistent with these data, QCM investigations showed that CA was readily adsorbed onto PAH or PSS, but the subsequent deposition of PAH onto a PAH/CA film or PSS onto a PSS/CA film resulted in no further mass being added onto the quartz crystal, similar to what was observed elsewhere for an invertase/poly(dimethyldiallylammonium chloride) system [258].

CA films were also prepared on a QCM crystal to determine the adsorption of BCA onto alternating PAH and PSS layers, as a PSS/BCA/PAH/BCA/PSS/BCA film (Figure 4-2a) and a BCA/PSS/BCA/PAH/BCA film (Figure 4-2b). However, for both cases, the rate of decrease in frequency slowed after repeated depositions of CA, which was again consistent with the $\zeta$-potential results. The quantity of BCA deposited onto PAH layers was greater than that deposited onto PSS layers, reflecting the stronger electrostatic attraction forces between the negatively charged BCA and the positively charged PAH. The $\Delta D:\Delta F$ ratios were consistently of the order $10^{-8}$ Hz$^{-1}$, which reflects that Equation 3-4 is valid for estimating the mass of polyelectrolyte being deposited onto the quartz crystal surface.
Figure 4-2. Real-time QCM investigations of the deposition of (a) PSS/BCA/PAH/BCA/PSS/BCA, (b) BCA/PSS/BCA/PAH/BCA, (c) [BCA/PSS/PAH]₂/BCA, and (d) [NCA/PSS/PAH]₂/NCA multilayers.

However, the deposition of BCA/PSS/PAH (Figure 4-2c) or NCA/PSS/PAH (Figure 4-2d) in repeating trilayers gave a better indication of consistent film formation in comparison to the films that were fabricated in Figures 4-2a or 4-2b. A frequency decrease of 226 ± 11 Hz per layer of NCA was observed for a [NCA/PSS/PAH]₂/NCA film (Figure 4-2d), which translates into an NCA coverage of 790 ± 30 ng cm⁻². This value is significantly greater than the single layer enzyme loading obtained from a covalent coupling of the BCA enzyme to a surface reported previously to a surface (less than 100 ng cm⁻²) [35, 217]. This value was also higher than the 280 ng cm⁻² of CA that was deposited as PEI/CA bilayers in prior work (the type of CA that was used was unspecified) [220].
Figure 4-3. (a) TEM image of the MS nanoparticles before deposition. SEM images of MS nanoparticles on silicon wafers after (b) one layer, (c) two layers, (d) three layers of coating. Scale bars in (b-d) are 2 µm.

To increase the quantity of enzyme being immobilized onto the films, MS particles with an average size of 115 nm (Figure 4-3a) were synthesized and used to load NCA in films of [MS/NCA/PAH]$_2$/MS/NCA onto either a glass plate or a silicon wafer (Figures 4-3 b, c, d). The quantity of NCA adsorbed in this case was determined from the difference in the UV absorbance of the NCA solution before and after contact with the glass substrate. Each coating of NCA was found to add 190 ± 20 µg cm$^{-2}$ of NCA to the MS layer on the film (Figure 4-4), which is approximately 140 times greater than the adsorption of NCA onto PAH.
These significantly increased enzyme loadings are indicative of the mesoporous nanoparticles with their high surface areas allowing for large polymer/protein loadings [251]. In comparison, the concentration of NCA in the supernatant did not change significantly during the fabrication of the \([\text{NCA/PSS/PAH}]_2\text{NCA}\) film, which is consistent with the lower quantities of enzyme adsorbed onto PAH as compared to the MS nanoparticles.

### 4.5 Multilayer Film Characterization

The total thickness of the multilayered protein films can significantly influence the mass transfer performance in membrane contactor applications. Ideally, the deposited polyelectrolyte layers will cover the pores of the top surface (typically 100 nm in diameter) to reduce pore wetting, but their thickness should not result in a significant resistance to the flow of carbon dioxide across the membrane. The total film thickness of the three enzyme layers in

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**Figure 4-4.** Quantity of NCA adsorbed onto each MS nanoparticle layer deposited onto the film.
addition to the precursor film can be calculated based on Equation 3-5. These film thicknesses are shown in Table 4-1:

**Table 4-1. Maximum polyelectrolyte film thicknesses**

<table>
<thead>
<tr>
<th>Polyelectrolyte Film</th>
<th>Maximum Film Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSS/BCA/PAH/BCA/PSS/BCA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>BCA/PSS/BCA/PAH/BCA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>[BCA/PSS/PAH]&lt;sub&gt;2&lt;/sub&gt;/BCA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>[NCA/PSS/PAH]&lt;sub&gt;2&lt;/sub&gt;/NCA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154 ± 8</td>
</tr>
<tr>
<td>[MS/NCA/PAH]&lt;sub&gt;2&lt;/sub&gt;/MS/NCA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>337 ± 67</td>
</tr>
</tbody>
</table>

<sup>a</sup>These results were obtained from QCM measurements. <sup>b</sup>This result was obtained from a cross-sectional SEM analysis of a fractured silicon wafer.

The thickness of the PSS/BCA/PAH/BCA/PSS/BCA and the BCA/PSS/BCA/PAH/BCA films are similar to the protein bilayer thicknesses and the PAH/PSS bilayer thicknesses that have been described elsewhere [220, 232]. The [BCA/PSS/PAH]<sub>2</sub>/BCA films were two to three times thicker than the PSS/BCA/PAH/BCA/PSS/BCA or the BCA/PSS/BCA/PAH/BCA films, which can be attributed to more BCA adsorbing onto a PAH precursor layer than onto a PSS precursor layer, as evidenced in the QCM analyses from Figure 4-2. The average frequency change was 9.2 ± 2.5 Hz when BCA was adsorbed onto PSS, which is approximately half of the 17 ± 5 Hz exhibited by the adsorption of BCA onto PAH.

The thickness of the [NCA/PSS/PAH]<sub>2</sub>/NCA films was four times greater than the equivalent thickness of the [BCA/PSS/PAH]<sub>2</sub>/BCA films. This reflects the greater mass of NCA and other proteins that
adsorb onto the quartz crystal at every deposition step (Figure 4-2). The [MS/NCA/PAH]$_2$/MS/NCA film is thicker again as a result of the addition of the MS nanoparticles, which are approximately 115 nm in diameter. A film thickness of approximately 337 ± 67 nm indicates that there is multilayer coverage of the nanoparticles on the surface of the silicon wafer.

4.6 Enzyme Activity Assays

Within experimental error, the resulting [BCA/PSS/PAH]$_2$/BCA films exhibited a uniform $p$-NP production rate of 0.10 ± 0.01 μmol min$^{-1}$ cm$^{-2}$. This $p$-NP production rate was irrespective of the number of BCA layers that were adsorbed (Figure 4-5). However, the PSS/BCA/PAH/BCA/PSS/BCA and the BCA/PSS/BCA/PAH/BCA films showed that the BCA adsorbed preferentially more onto PAH than PSS. The similar $p$-NPA hydrolysis rates (instead of an increased enzymatic activity upon the deposition of more enzyme layers [237]) suggest that the hydrophilic PAH and PSS layers were sufficiently non-porous to limit the diffusion of the large hydrophobic $p$-NPA molecules to the lower layers of immobilized BCA.
Figure 4-5. Enzymatic activity of [BCA/PSS/PAH]$_2$/BCA, PSS/BCA/PAH/BCA/PSS/BCA and BCA/PSS/BCA/PAH/BCA films in the hydrolysis of p-NPA.

However, CO$_2$ is a small uncharged molecule that will be able to penetrate the polyelectrolyte multilayers more readily. Thus, it is necessary to evaluate the contribution of each immobilized CA layer to the overall total enzymatic activity using CO$_2$ hydration assays. Glass slides were prepared with up to three adsorbed layers of CA as [CA/PSS/PAH]$_2$/CA films and assayed for their CO$_2$ hydration activities.

A calibration curve was first prepared for determining the CO$_2$ hydration activity of the free NCA in a saturated CO$_2$ solution. The activity of the free NCA is shown to be approximately 2.7 μmol min$^{-1}$ μg$^{-1}$ of NCA (Figure 4-6). However, the specific activity of the NCA decreased to 2.1 μmol min$^{-1}$ μg$^{-1}$ after it was stored at pH 12 over a period of 72 h at 4°C (Figure 4-6). A number of other researchers have also shown that CA loses activity upon exposure to high pH [2, 55]. However, the residual activity recorded here (78%) is significantly greater than that observed in those earlier works. Sharma and
Bhattacharya [2] observed residual activities of between 24 and 57% across four CA varieties after exposure for only 6 h at pH 10, presumably at ambient temperature. Similarly, Zhang et al. [55] similarly observed a residual activity of 63% for a commercial enzyme when tested at pH 10.5 and 4°C.

Figure 4-6. CO₂ hydration rate as a function of the quantity of free NCA added to solution. The filled diamond symbols (♦) indicate the rate for fresh NCA solution, while the open squares (□) indicate the rate of hydration for NCA that was incubated for 72 hours at pH 12 and 4°C.
Figure 4-7 shows that the overall CO$_2$ hydration activity of the [BCA/PSS/PAH]$_2$/BCA film was approximately 0.52 ± 0.09 μmol min$^{-1}$ cm$^{-2}$ per layer of adsorbed BCA. This CO$_2$ hydration activity is approximately 5 times greater than the $p$-NPA hydrolysis activity, which is consistent with the CO$_2$ hydration to $p$-NPA hydrolysis activity ratios that have been reported elsewhere [163]. This activity ratio reflects the limitations of the $p$-NPA diffusion within the film. Moreover, in contrast with the $p$-NPA analyses, the CA activity increases linearly with the deposition of further layers when hydrating CO$_2$, regardless of the type of CA that was used (Figure 4-7). This linear trend indicates that the dissolved CO$_2$ is able to readily penetrate through the PAH/PSS bilayers to the lower levels of the film for reaction. It is probable that the activity eventually plateaus with increasing CA layers because of diffusion rate limitations, as was observed in activity assays of immobilized peroxidase enzymes [237].
The CO₂ hydration activity of the film prepared with NCA was observed to be 2.6 ± 0.7 μmol min⁻¹ cm⁻² per layer of adsorbed NCA, which was approximately 5 times greater than that of the BCA film. The specific activity of the NCA in terms of the mass of adsorbed NCA was 3.5 μmol min⁻¹ μg⁻¹, which is comparable to the specific activity of the free NCA at 2.7 μmol min⁻¹ μg⁻¹ within experimental error. This comparable activity indicates that there is no significant loss of activity upon immobilization onto the plate.

The addition of the MS nanoparticles onto the glass plate further boosts the CO₂ hydration activity to 13 ± 4 μmol min⁻¹ cm⁻² per layer of adsorbed NCA. However, given that the adsorption of NCA was as high as 190 μg cm⁻² NCA (Figure 4-4), this CO₂ hydration activity translates to a specific NCA activity of 0.067 μmol min⁻¹ μg⁻¹. This specific activity is a decrease of 97.5% in comparison with the specific activity of the free enzyme at 2.7 μmol min⁻¹ μg⁻¹ NCA. Therefore, there is a loss of enzymatic activity when the NCA is immobilized onto the nanoparticles, while the specific activity is seen to increase if NCA is adsorbed in a polyelectrolyte film. Similar losses in enzymatic activity have been reported previously for both nonporous nanoparticles [55] and porous nanoparticles [266]. In the present case, the decline in the specific enzyme activity is likely to be the result of the added diffusional resistance for the CO₂ molecules [266] when entering the pores of the MS nanoparticles.

4.7 Membrane Characterization

When coated on the surface of a porous polypropylene membrane, the MS particles continue to outperform a simple multilayer film, in this case by a factor of four (Figure 4-9) at an average rate of 19 ± 4 μmol cm⁻² min⁻¹ per layer in comparison with the multilayer film performance at 5.3 ± 0.8 μmol cm⁻² min⁻¹ per layer. In the enzymatic activity on the membrane is approximately double relative to the flat glass plate (Figure 4-8). This increased enzymatic activity is consistent with the data in
Figure 4-4, which also shows a doubling in NCA adsorption from 190 to 440 μg cm⁻² when the plate is replaced with a membrane. This increase in enzyme activity probably reflects some penetration of the enzyme layers into the porous structure.

![Graph showing CO₂ hydration activity as a function of the number of NCA layers on the PP membranes.](image)

**Figure 4-8.** CO₂ hydration activity as a function of the number of NCA layers on the PP membranes.

These hydration rates compare well to the flux rates determined for CO₂ absorption within membrane contactors described in the literature. Flux rates for the absorption of CO₂ into monoethanolamine through a porous PP contactor range from 1.5 to 30 μmol cm⁻² min⁻¹ [268, 269]. The CO₂ flux is generally lower for solvents with slower reaction kinetics such as potassium carbonate, where promotion with CA would be of value. Capannelli *et al.* [270] report a flux of approximately 4 μmol cm⁻² min⁻¹ for a 1 M K₂CO₃ solution, versus 50
μmol cm⁻² min⁻¹ for the same concentration of piperazine and 30 μmol cm⁻² min⁻¹ for monoethanolamine.

It can be observed that the incubation of the adsorbed NCA for 72 h at pH 12 leads to a loss of activity, but 70 to 80% of the residual activity is retained, in comparison with the adsorbed NCA that was incubated at pH 7.2 (Figure 4-9). This activity retention is consistent with the data recorded for the free enzyme (Figure 4-6), indicating that the enzyme is more tolerant of high pH conditions than other strains that have been presented in the literature [2, 55].

![Figure 4-9](image)

**Figure 4-9.** The enzymatic activity of the immobilized membrane films both as prepared (grey bars) and after incubation for 72 h at pH 7.2 or pH 12 and 4°C. These films were prepared as [NCA/PSS/PAH]₂/NCA films (denoted as NCA) and [MS/NCA/PAH]₂MS/NCA (denoted as MSNCA), respectively.

SEM images of the PP membrane surfaces and cross sections before and after LbL treatment (Figure 4-10) show clear evidence of the multilayer films and MS spheres on the surface. Figure 4-10b shows that the [NCA/PSS/PAH]₂/NCA layers form a flat dense film [241] that is akin to the formation of nanofiltration-type barriers caused by the interactions between PAH and PSS [242]. Figures 4-10c and 4-10d also
show that the MS spheres assemble uniformly onto the membrane surface. However, there is little evidence of deep penetration into the porous substructure (Figures 4-10e and 4-10f). The multilayer film thicknesses on the membrane surface are of the order of 300-500 nm, consistent with that observed for the flat plate (Table 4-1), which means that the mass transfer resistance through the membrane itself should not be significantly compromised.

Figure 4-10d indicates that there has been little degradation of the MS spheres after storage at pH 12 for 72 h. However, it should be noted that silica materials will dissolve in an alkaline environment over a long period of time. The instability of silica materials at high pH could limit the use of the MS nanoparticles in carbon capture applications operating under high pH conditions.

**Figure 4-10.** The PP membrane surface (a) before LbL treatment, (b) after LbL treatment with [NCA/PSS/PAH]₂NCA, (c) after LbL treatment with [MS/NCA/PAH]₂MS/NCA and storage at pH 7.2 for 72 h, (d) after LbL treatment with [MS/NCA/PAH]₂MS/NCA and storage at pH 12 for 72 h. The PP membrane cross-section (e) after LbL treatment with [NCA/PSS/PAH]₂NCA, (f) after LbL treatment with [MS/NCA/PAH]₂MS/NCA. The black bars in (e) and (f) indicate the approximate thickness of the LbL films.
It is well known that the pores of the membrane must remain filled with gas to maintain effective mass transfer rates in a membrane contactor [95], and a reduction of the local pore diameter could assist in preventing the wetting of the pore volume with solvent [130, 132]. Conversely, the deposition of CA and other polyelectrolytes on the membrane surface could reduce the contact angle and so enhance wetting. In the present case, the pressure required to force water through the membrane (the liquid entry pressure) was recorded experimentally, as an indication of these competing effects (Table 4-2):

**Table 4-2.** Characterization of the PP membrane before and after LbL treatment

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Contact Angle (°)</th>
<th>Liquid Entry Pressure (kPa)</th>
<th>Water Flux (kg m⁻² h⁻¹) at 575 kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP (clean)</td>
<td>96 ± 2</td>
<td>&lt; 525</td>
<td>9.4 ± 0.6</td>
</tr>
<tr>
<td>PP (LbL with NCA)</td>
<td>60 ± 2</td>
<td>&lt; 550</td>
<td>2.25 ± 0.08</td>
</tr>
<tr>
<td>PP (LbL with MSNCA)</td>
<td>52 ± 1</td>
<td>&lt; 575</td>
<td>0.53 ± 0.02</td>
</tr>
</tbody>
</table>

Table 4-2 indicates that the LbL treatment caused the membrane surface to become more hydrophilic, as evidenced by the contact angle measurements. However, an increased pressure was required to force water through the thin film that was fabricated on the membrane, and the water flux was also observed to decline, particularly after LbL treatment with the MSNCA film. This confirms that the average pore size has been reduced significantly, as is evident from the images in Figure 4-10. The tighter pore size will reduce pore wettling, but may also increase the resistance to the mass transfer of carbon dioxide across the membrane. A compromise between these two competing effects will be required.

### 4.8 Conclusions

This work has shown that carbonic anhydrase can be readily applied to the surface of a porous polymeric membrane as a uniform
thin film. The use of LbL techniques leads to enzyme loadings that are significantly greater than those previously obtained using covalent coupling. The diffusion of CO$_2$ through these multilayers was unhindered, as evidenced by the uniform increase in CO$_2$ hydration activity with each successive CA layer. The use of a thermostable microbial enzyme (NCA) leads to higher enzyme loadings and to greater enzymatic efficiency. The addition of MS nanoparticles to the polyelectrolyte film prior to enzyme adsorption increases the CO$_2$ hydration rates by eightfold relative to the bovine enzyme film. However, the specific activity of the NCA decreased sharply upon immobilization onto the MS nanoparticles, which may be indicative of diffusional resistances for CO$_2$ molecules entering the pores of the nanoparticles for reaction. It should also be noted that silica materials can dissolve in an alkaline environment over time. This will further limit the use of the MS nanoparticles in carbon capture applications which tend to operate at high pH.

While it can be observed that the overall CO$_2$ hydration rates increased when the enzyme was adsorbed onto a membrane, the flux through the membrane declined, which may lead to additional mass transfer resistance in a full scale membrane module. These results show potential towards the development of new membrane materials for use as membrane contactors in CO$_2$ absorption processes. However, the activity of these membranes was only quantified in a stirred solution pre-saturated with carbon dioxide, where mass transfer limitations are avoided. The results need to be validated by work at a larger scale in a gas-liquid membrane contactor format at realistic pH levels and over a longer period of time, which will be the focus of our ongoing work.
Chapter 5: In Situ Layer-by-Layer Assembly-Based Carbonic Anhydrase-Coated Hollow Fiber Membrane Contactors for Rapid CO2 Absorption

5.1 Chapter Perspective

Chapter 4 illustrated the fabrication of uniform films containing carbonic anhydrase (CA) onto flat nonporous and porous surfaces alike, which makes the layer-by-layer (LbL) technique for preparing membrane contactors to absorb carbon dioxide a potentially useful solution for carbon capture applications. However, hollow fiber membrane configurations are more space efficient than flat sheet configurations, which led to the work in Chapter 5. Chapter 5 focuses on the fabrication of these thin films on hollow fiber membranes under both static and dynamic adsorption conditions, where static adsorption was found to be the most effective method for increasing the absorption rates of carbon dioxide. In addition, porous polypropylene (PP) and nonporous polydimethoxysilane (PDMS) hollow fibers were compared to determine the change in the mass transfer resistance, as it is known that the pores in the PP membrane will become wetted and lead to a reduced mass transfer rate over time, while the nonporous PDMS hollow fibers face no issues with wetting at all.

This chapter was published as J. K. J. Yong, G. W. Stevens, F. Caruso, S. E. Kentish, In Situ Layer-by-Layer Assembly-Based Carbonic Anhydrase-Coated Hollow Fiber Membrane Contactors for Rapid CO2 Absorption, J. Membr. Sci. 514 (2016) 556-565.
5.2 Abstract

The use of potassium carbonate as a solvent for the absorption of carbon dioxide is constrained by slow absorption kinetics, which hinders the overall rate of mass transfer. In this work, the reaction rate is promoted by the electrostatic adsorption of carbonic anhydrase (CA) onto the surface of both a porous polypropylene (PP) and a non-porous polydimethoxysilane (PDMS) hollow fiber membrane via layer-by-layer (LbL) assembly. The rate of CO2 absorption into K2CO3 is increased approximately threefold when CA is adsorbed onto the PP membrane surface, while the absorption rate of the modified PDMS membrane was slightly lower, within 70–90% of the PP values. The results show that the ultrathin CA films are assembled mainly on the surface of the membranes and do not penetrate into the depth of the membrane pores. The CO2 hydration is enhanced in all cases, and the wetting of the porous PP membranes is reduced significantly by the pore blockage induced by the LbL adsorption of the polyelectrolytes.
5.3 Introduction

Membrane gas absorption (MGA) has been investigated as an alternative to conventional packed towers which use solvent absorption for the capture of CO₂ from the flue gas streams produced by fossil fuel-fired power plants. This approach provides higher surface-to-volume ratios and thus a smaller equipment footprint [6]. These contactors also provide a better mass transfer efficiency in comparison to the absorption towers [87, 91]. The material requirements for the construction of membrane modules are also significantly less [84].

In a membrane-based gas-liquid contactor process, a hydrophobic membrane material acts as a barrier between the incoming flue gas and the absorption solvent [271]. This membrane prevents the solvent from entering the gas phase while allowing the gas to permeate through the membrane and be absorbed into the solvent. At the industrial scale, the solvents that are most commonly used for absorption are alkanolamines such as monoethanolamine (MEA) or diethanolamine (DEA) [1]. However, these alkanolamines are highly corrosive [1, 7] and are readily degraded in the presence of oxygen [18], so that their absorption capacity will decrease over time. Moreover, the thermodynamic stability of the carbamates also dictates that the desorption of CO₂ from the solvent for solvent reuse and CO₂ storage will require an extremely high energy penalty [17].

To reduce the energy penalty from the desorption process, alternative solvents such as potassium carbonate (K₂CO₃) may be used, as has been demonstrated in the case of the Benfield absorption process [79]. However, the absorption kinetics for K₂CO₃ are far slower than that of MEA or DEA [6, 65]. Increased CO₂ absorption kinetics within carbonate or bicarbonate solvents may be observed upon the addition of natural enzymes such as carbonic anhydrase (CA) into the solvent [264], as this enzyme is known to catalyze the conversion of CO₂ into HCO₃⁻ extremely rapidly [176].
CA has been shown to retain its activity upon covalent immobilization onto the surface of a membrane [51]. While such immobilization may provide good protein stability [52], it is a time consuming process that requires multiple reaction steps and makes use of toxic chemicals [51, 272]. In comparison, the electrostatic adsorption of enzymes onto membrane surfaces via the deposition of alternatively charged polyelectrolytes, a process referred to as layer-by-layer (LbL) technology, proceeds relatively rapidly, makes use of much less toxic substances and also makes use of small quantities of enzyme (10 to 1000 \( \mu g \) m\(^{-2}\)) in film fabrication [237]. The mass production of enzymes from recombinant cell lines at moderate cost [175] would then further aid in the development of immobilized enzyme hollow fiber membrane contactors for \( CO_2 \) capture, given that only relatively small quantities of the enzyme are required to improve the absorption rates of \( CO_2 \) into \( K_2CO_3 \) [47].

The immobilization of proteins onto flat sheet microporous membranes via LbL technology has been investigated previously [234, 237, 273]. However, these membranes have been used mainly for catalyzing reactions that occurred within a singular liquid phase, not in a biphasic gas-liquid absorption system. In a membrane-based gas-liquid absorption system, the key to maintaining an effective mass transfer rate lies in ensuring that the pores remain filled with gas rather than solvent. Wetted pores can contribute approximately 30\% of the overall mass transfer resistance [129]. A degree of wetting as little as 5\% can reduce the overall mass transfer rates by as much as 20\% [128]. The ability of a membrane to withstand the breakthrough of a liquid through its pores is quantified by a parameter known as the liquid breakthrough pressure (LBP), which is the pressure that is required to force a liquid through the pores of the membrane. The Laplace-Young equation (Equation 2-26) relates the LBP of a membrane to the hydrophobicity and the pore size of the membrane [130], as has been covered in Chapter 2.
The adsorption of polyelectrolytes such as polyallylamine hydrochloride (PAH) and polystyrene sulfonate (PSS) onto a porous ultrafiltration membrane surface has been shown to reduce the pore size of the membrane to that comparable to a nanofiltration membrane [242, 243]. Indeed, LbL film assembly has previously been used for converting ultrafiltration membranes into nanofiltration and forward osmosis membranes in both flat sheet [242] and hollow fiber configurations [274, 275]. The reduction in the overall pore size may be useful in reducing the effect of pore wetting on MGA operation. However, the increase in membrane hydrophilicity upon adsorption of these charged polyelectrolytes may provide a competing effect that reduces the LBP by reducing the contact angle of the membrane surface.

Our previous work has shown that the fabrication of CA-containing polyelectrolyte films via LbL assembly on a flat sheet polypropylene membrane was useful in increasing the rate of CO₂ hydration into HCO₃⁻ [273]. However, it is to the best of our knowledge that there have been no investigations of using LbL film assembly onto hollow fiber membranes for this purpose.

In this work, we focus on the assembly of ultrathin films incorporating CA onto the surface of microporous polypropylene (PP) or nonporous polydimethoxysilane (PDMS) hollow fiber membranes through the LbL assembly technique. We apply the ultrathin coating to the fibres in situ within an assembled module. The modified membranes are then tested for their CO₂ separation capabilities. We show that the mass transfer coefficient approximately doubles that of a biologically inactive polyelectrolyte film, due to reductions in pore wetting. Upon the incorporation of CA into the film, the mass transfer coefficient increases further, for both the porous PP and the nonporous PDMS membranes.
5.4 SEM Microscopy of the Hollow Fiber Membranes

Figure 5-1. SEM images of hollow fiber membrane surfaces from (a) unmodified PDMS, (b) PDMS after static LbL adsorption of a PEI/PSS/PAH/[PSS/PAH/NCA]₂ film, (c) unmodified PP, (d) PP after static LbL adsorption of a PEI/PSS/PAH/[PSS/PAH/NCA]₂ film, (e) PP after adsorption of a PEI/PSS/PAH/[PSS/PAH/NCA]₂ film in permeation mode, (f) PP with two NCA trilayers applied in pump through mode (pump flow rate 20 mL/min, adsorption time 5 min).

In our previous work, we showed that it was possible to obtain uniform coverage of a flat sheet PP membrane with a PEI/PSS/PAH/[PSS/PAH/NCA]₃ coating applied using a simple paintbrush [273]. In this work, SEM images of the PDMS membrane before and after adsorption (Figures 5-1a and 5-1b) indicate that the surface of the PDMS layer is evenly coated with the polyelectrolyte solution, probably because it was already smooth and non-porous prior to adsorption. However, the coating of the PP hollow fiber membranes in situ was less successful (Figures 5-1c to 5-1f), with some defects in the film clearly evident. These defects may reflect the greater pore size of these PP membranes (0.2 µm versus 0.1 µm) or the more indirect coating method that was utilized.
A close inspection of the SEM images from the PP membranes coated in a pump through mode indicated that better coverage was achieved through longer adsorption times and lower adsorption flowrates. However, the three approaches to coating in general (pump through mode, static mode, permeation mode) appeared to give comparable coatings, with some evidence of a slightly more complete coating with the permeation mode (Figure 5-1e).

**Figure 5-2.** A cross section of PP hollow fiber membranes (a) before LbL treatment, (b) after LbL treatment in static mode, (c) after LbL treatment
in permeation mode and PDMS hollow fiber membranes (d) before LbL treatment and (e) after LbL treatment. Scale bars = 2 µm.

Figure 5-2 shows that there is little difference in the cross-sectional morphology of the untreated PP or PDMS membranes (Figures 5-2a and 5-2d) in comparison with membranes that have been coated with multiple layers of polyelectrolytes (Figures 5-2b, 5-2c and 5-2e). There is no evidence of polyelectrolyte permeation into the bulk of the hollow fiber membranes with all the enzyme activity concentrated at the membrane surface. There is no evidence of a thick dense polyelectrolyte layer building up on the surface of the membrane, which shows that the films are extremely thin and will not contribute significantly to an increased membrane mass transfer resistance (Figures 5-2b and 5-2e). The porosity of the substructure also appears to be relatively unchanged, providing little resistance to CO₂ mass transfer. The cross section image of the PP membrane in permeate mode shows a polyelectrolyte layer of less than 1 µm thickness (Figure 5-2c), which is consistent with our previous observations of polyelectrolyte film thickness when coated onto planar membrane coupons [273].

5.5 The Influence of Polyelectrolyte Layer Buildup onto PP Fibers on the Absorption of CO₂

The overall mass transfer coefficients for CO₂ absorption through PP hollow fibers into 30 wt% K₂CO₃ can be calculated from Equation 2-4. For an unmodified contactor, this mass transfer coefficient does not vary significantly with the Reynolds number, with a value of approximately 1.7 × 10⁻⁵ m s⁻¹. The lack of dependence on Reynolds number suggests that the liquid phase is not rate controlling, but rather, membrane wetting contributes significantly to the membrane mass transfer coefficient.

The addition of pure polyelectrolyte films containing no enzyme in pump through mode leads to a significant increase in the mass transfer
rate (Figure 5-3). The addition of four trilayers (PEI/[PSS/PAH]₄) is more effective than the additions of two trilayers (PEI/[PSS/PAH]₂), with the mass transfer coefficient approximately doubling in this case to between 2 and 4 × 10⁻⁵ m s⁻¹. This increase in the mass transfer coefficient suggests that the successive addition of LbL films is restricting membrane wetting by reducing the pore size at the surface. Even though the adsorption of the film onto the membrane surface causes the membrane surface to become more hydrophilic [273], the coating is able to reduce liquid penetration into the pores. The increased dependence of the mass transfer coefficient on the Reynolds number also suggests that the role of membrane resistance is declining relative to the liquid phase resistance.

**Figure 5-3.** Overall mass transfer coefficients as a function of the shell side Reynolds number during gas absorption at 25°C. Data is shown for an unmodified PP membrane and upon the adsorption of either a pure polyelectrolyte film (PEI/[PSS/PAH]₄) or an enzyme film (PEI/PSS/PAH/[PSS/PAH/NCA]ₓ where x = 1, 2 or 3) in pump through mode. Adsorption flow rate = 120 mL min⁻¹, adsorption time = 5 min.
This reduction in pore wetting can be confirmed by application of Equations 2-12 to 2-24. For PP fibers that were not coated, the reaction enhancement factor is calculated to be 1.06 (Equations 2-13 to 2-15), which is in good agreement with results published elsewhere [6]. With this enhancement factor known, the pore wetting can be estimated as 4% for these unmodified PP fibers (Equations 2-18 to 2-24). Upon the application of the PEI/[PSS/PAH]_2 film, the pore wetting reduces to 2.3%, while the addition of four trilayers (PEI/[PSS/PAH]_4) reduces it further to 0.5% (see Table 5-1).

However, the adsorption of a single trilayer of NCA within these polyelectrolyte films increases the mass transfer coefficient to a much greater extent throughout the range of solvent flow rates (Reynolds numbers) that were investigated, as shown in Figure 5-3. The adsorption of more than one trilayer onto the membrane causes a further minor increase in the overall mass transfer coefficient. This increased mass transfer coefficient reflects both a higher NCA loading and an increase in the surface coverage of the membrane.

The pore wetting factors determined in the absence of any enzyme can now be used to calculate the enhancement factors for these enzyme containing systems, again using Equations 2-18 to 2-24 (Table 5-1). There is a clear increase in the enhancement factor when the number of NCA deposition cycles is increased from 1.9 with one trilayer to 6.9 with three trilayers containing NCA (Table 5-1). This increased enhancement factor confirms that the addition of more enzyme layers is both increasing reaction rates as well as reducing pore wetting.
Table 5-1. The contribution of the membrane mass transfer resistance to the overall mass transfer resistance at ambient temperature.

<table>
<thead>
<tr>
<th>Membrane Coating</th>
<th>Adsorption Mode</th>
<th>Enhancement Factor</th>
<th>Pore Wetting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>None</td>
<td>None</td>
<td>1.06</td>
</tr>
<tr>
<td>PDMS</td>
<td>None</td>
<td>None</td>
<td>1.06</td>
</tr>
<tr>
<td>PP</td>
<td>0.05 vol% in solution</td>
<td>None</td>
<td>8.4</td>
</tr>
<tr>
<td>PP</td>
<td>0.2 vol% NCA in solution</td>
<td>None</td>
<td>12</td>
</tr>
<tr>
<td>PP</td>
<td>PEI/[PSS/PAH] (_2) (no NCA)</td>
<td>Pump through</td>
<td>1.06</td>
</tr>
<tr>
<td>PP</td>
<td>PEI/[PSS/PAH] (_4) (no NCA)</td>
<td>Pump through</td>
<td>1.06</td>
</tr>
</tbody>
</table>

**PEI/PSS/PAH/[PSS/PAH/NCA] \(_2\) film**

<table>
<thead>
<tr>
<th>Polyelectrolyte flow rate (mL/min)</th>
<th>Adsorption time per layer (min)</th>
<th>NCA deposition cyc.es (x)</th>
<th>Adsorption Mode</th>
<th>Enhancement Factor</th>
<th>Pore Wetting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>120</td>
<td>5</td>
<td>1</td>
<td>Pump through</td>
<td>1.9</td>
</tr>
<tr>
<td>PP</td>
<td>120</td>
<td>5</td>
<td>2</td>
<td>Pump through</td>
<td>4.7</td>
</tr>
<tr>
<td>PP</td>
<td>120</td>
<td>5</td>
<td>3</td>
<td>Pump through</td>
<td>6.9</td>
</tr>
<tr>
<td>PP</td>
<td>50</td>
<td>5</td>
<td>2</td>
<td>Pump through</td>
<td>4.9</td>
</tr>
<tr>
<td>PP</td>
<td>20</td>
<td>5</td>
<td>2</td>
<td>Pump through</td>
<td>4.9</td>
</tr>
<tr>
<td>PP</td>
<td>20</td>
<td>15</td>
<td>2</td>
<td>Pump through</td>
<td>5.0</td>
</tr>
<tr>
<td>PP</td>
<td>20</td>
<td>30</td>
<td>2</td>
<td>Pump through</td>
<td>5.2</td>
</tr>
<tr>
<td>PP</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>Static</td>
<td>5.3</td>
</tr>
<tr>
<td>PDMS</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>Static</td>
<td>6.2</td>
</tr>
</tbody>
</table>
Figure 5-4. Overall mass transfer coefficients as a function of the shell side Reynolds number during gas absorption at 25°C; after the fabrication of a pure polyelectrolyte film (PEI/[PSS/PAH]₄) or an enzyme film (PEI/PSS/PAH/[PSS/PAH/NCA]₂) on a PP membrane contactor in pump through mode. The pump flow rate is 20 mL/min, while the adsorption time is varied as shown.

Figure 5-4 indicates that there is also a clear increase in the mass transfer coefficient when the polyelectrolyte solutions are pumped through the module for longer periods. There is also a slight increase in the overall mass transfer coefficient when the flow rate that is used for adsorption is lower, which can be attributed to a reduced shear rate that is experienced by the polyelectrolytes (Figure 5-5).
Figure 5-5. Overall mass transfer coefficients as a function of the shell side Reynolds number during gas absorption at 25°C; after the fabrication of a pure polyelectrolyte film (PEI/[PSS/PAH]_4) or an enzyme film (PEI/PSS/PAH/[PSS/PAH/NCA]_2) on a PP membrane contactor. The adsorption time is 5 minutes, while the pump circulation flowrate is varied from zero (static mode) to between 20 and 120 ml/min (pump through mode).

A reduced liquid shear rate on the membrane surface allows for better dynamic adsorption of the polyelectrolytes, as is evidenced in research regarding the adsorption of foulants onto a membrane surface [276]. A static adsorption, where there was no flow during the 5 minute adsorption period and thus no shear, provided the greatest increase in the mass transfer coefficient (Figure 5-5). Similar increases in the enhancement factor are also observed (Table 5-1).

5.6 Investigating the Effects of Membrane Drying on the Mass Transfer Performance

It can be observed that the fibers dried with N₂ after static adsorption prior to conducting the solvent absorption experiments
behaved in a comparable manner to those that had not been dried (Figure 5-6). These comparable mass transfer coefficients indicate that the polyelectrolyte adsorption process did not itself wet the membrane pores and also that the NCA activity is only slightly reduced after this drying process. In contrast, when the polyelectrolyte solutions are forced to permeate through the membrane pores, the overall mass transfer coefficients fall to 59 ± 3% of the static mode values, even after drying with N₂.

Figure 5-6. The effects of membrane wetting on the mass transfer of CO₂ in the PP hollow fibers at 25°C. PEI/PSS/PAH/[PSS/PAH/NCA]₂ films were fabricated onto the membranes.

The lower mass transfer coefficients arising from coating in permeation mode could arise because a thicker film is formed (Figure 5-2c); because the film itself blocks some of the pores; or because the passage of the polyelectrolyte solution through the pores renders them more hydrophilic and hence more susceptible to wetting even after drying with N₂. The fabrication of polyelectrolyte films onto hollow fiber
membranes for nanofiltration purposes were reported by Menne *et al.* [275] to be more uniform and reduced the pore size more significantly than in pump through mode, which resulted in a better salt retention but a lower flux performance. In the case of the membrane contactors that were investigated in this study, the fibers that were coated in permeation mode were still found to provide higher mass transfer rates than the unmodified membrane (Figure 5-6) because of adsorbed NCA providing an enhanced chemical reaction rate.

### 5.7 A Comparison of the Free Enzyme with the Immobilized Enzyme

The performance of a PP membrane contactor where the fibers had been coated with two CA trilayers was also compared with the use of free CA (0.2% v/v) in the potassium carbonate solvent (Figure 5-7). Two concentrations of free CA were evaluated, 0.05% v/v and 0.2% v/v, corresponding to a total enzyme mass within the module at any time of 0.1 mg and 0.4 mg, which corresponds to enzyme concentrations of 75 mg/L or 300 mg/L in the module. The quantity of CA immobilized via static adsorption in 2 trilayers within the hollow fiber module can also be estimated to be approximately 0.1 mg, based on the activity and adsorption data provided in our previous work [273]. It can be observed that the overall mass transfer coefficient is approximately the same for the two cases where 0.1 mg of enzyme was used whether it was free or immobilized.
Figure 5-7. Overall mass transfer coefficients as a function of the shell side Reynolds number during gas absorption at 25°C for (i) an unmodified PP contactor (ii) an unmodified PP contactor with the addition of 0.2 vol% free NCA and (iii) a PP contactor coated with a PEI/PSS/PAH/[PSS/PAH/NCA]₂ film in static mode with an adsorption time of 5 min per layer; as a function of the solvent Reynolds number.

However, the module with free CA also suffers from greater pore wetting as discussed above. The enhancement factor is actually greater for the free CA case, as it needs to counteract this increase in pore wetting to achieve the same mass transfer rate. Indeed, the enhancement factor for the 0.05% v/v solvent is calculated as 8.4, somewhat larger than that for the immobilized case of 5.3 (Table 5-1 and Figure 5-8). While possibly within the range of experimental error, this may suggest a slight loss in enzyme activity upon immobilization, although we observed no such loss in our previous work using planar membrane surfaces [273].
Figure 5-8. The influence of the mass of immobilized or free enzyme on the enhancement factor of the CO$_2$ hydration reaction. The lines that are drawn are solely for illustration purposes and may not reflect the enhancement factor trends.

As shown in Figure 5-8, the enhancement factor increases with the enzyme loading but then plateaus at higher loadings for the free enzyme case. Similar limitations have been observed in earlier work by Thee et al. [47], where high enzyme dosages of 9.1 μM (approximately 270 mg L$^{-1}$) resulted in enzyme flocculation within the solvent and rendered the enzyme less effective. The enhancement factor with an unmodified PP membrane in the presence of free NCA (0.2% v/v) was the greatest recorded at 12.

5.8 A Comparison Between the PP and the PDMS Membrane Performance

The overall mass transfer coefficient of the unmodified PDMS membrane is comparable to that of the unmodified PP membrane, which implies that the impact of the dense non-porous PDMS layer is comparable to the impact that is provided by the membrane wetting in the PP system. This result is consistent with what has been reported by
Wang et al. [128], who observed that a degree of wetting as little as 5% could reduce the overall mass transfer rates by 20%. Adding the pure PEI/[PSS/PAH]₄ polyelectrolyte film to the PP membrane reduces this wetting as described above, so that this system outperforms the PDMS membrane in the absence of enzyme. Under these circumstances, the resistance of the dense non-porous PDMS layer is greater than that of an applied polyelectrolyte film. Differences in the porous membrane support structure may also play a minor role in differentiating the performance of these two membrane systems.

For both the porous PP and the PDMS membrane contactors, the mass transfer coefficient increases with an increased absorption temperature as reported elsewhere [6, 203] (Figure 5-9). The mass transfer coefficient is observed to be higher for the CA-coated porous PP fibers than the CA-coated PDMS fibers at all temperatures. Furthermore, the dependence upon the Reynolds number is less for the PDMS fibers than it is for the PP fibers. These results reflect the fact that the non-porous PDMS layer adds an additional resistance that does not change with the Reynolds number [134].
Figure 5-9. Influence of temperature on the absorption of CO₂ into K₂CO₃ as a function of the solvent Re. The data shown is representative of PEI/PSS/PAH/[PSS/PAH/NCA]₂ films coated onto the PP/PDMS hollow fibers in static mode with an adsorption time of 5 min per layer. Mass transfer data for the unmodified PDMS hollow fibers at 25°C is shown as a baseline scenario.

However, these experiments were conducted over a short period of time (approximately 6 hours). The PP membrane is likely to become further wetted over a longer period of time. This wetting will cause the mass transfer coefficient for this module to decrease because of an increased membrane mass transfer resistance. It is thus quite likely that the PP module will end up performing less efficiently than the PDMS membrane. Such behavior has been shown in long-term experiments conducted by Chabanon et al. [137], who compared the performance of PP and PDMS membrane contactors over a period of 50 days. However, the overall mass transfer coefficients for this PDMS membrane are still much greater than the mass transfer coefficients exhibited by the unmodified membranes.
In contrast with the PP membranes, the PDMS membranes have a dense nonporous PDMS layer on the membrane surface and thus will not experience any wetting. In this case, the best estimate of the membrane mass transfer coefficient can be estimated for the known carbon dioxide permeability of PDMS in the presence of water at 4500 Barrer $[277]$. This leads to a membrane mass transfer coefficient of $7.5 \times 10^{-3}$ m s$^{-1}$ for a PDMS thickness of 0.5 µm. The resulting reaction enhancement is comparable to that achieved with the PP system (Table 5-1).

5.9 Conclusions

The coating of a polyelectrolyte film (with or without NCA) onto microporous PP membranes in a static or pump through mode results in a reduction in pore wetting even though the membrane surface has been made more hydrophilic. The adsorption of the film occurs mainly on the membrane surface and does not result in any significant internal pore blockage. The reduction in pore wetting is beneficial in increasing mass transfer rates. The inclusion of CA within the film further promotes the absorption of CO$_2$ for both porous PP and dense PDMS hollow fiber membranes, leading to a substantial increase in the reaction enhancement factor. Adsorption was found to be slightly more effective at lower adsorption flow rates and higher adsorption times, and NCA surface coverage was also found to be more effective with the adsorption of more polyelectrolyte layers. The overall mass transfer coefficient was found to be similar to that observed when an equivalent amount of free NCA was used in solution. However, after accounting for pore wetting, this suggested that immobilization had indeed caused a slight loss in enzyme activity. While the performance of the coated PP fibers was slightly better than that of the equivalent PDMS fibers, the mass transfer coefficient exhibited by the porous PP membranes will experience a decline over time because of longer term membrane pore
wetting. From this perspective, the use of the non-porous PDMS system is likely to be the better option over long time frames.

The surface coverage of the film on the PP membrane surface was not completely uniform. The surface coverage appears to be better when the polyelectrolyte solution is forced to permeate through the membrane pores, which is in agreement with results that were reported elsewhere [275]. However, this latter permeation technique results in a reduced CO₂ mass transfer coefficient, which could arise either from pore blockage or because the resulting pore structure is more hydrophilic and hence more prone to membrane wetting.

Importantly, the film is applied in situ to fibers that have already been assembled within a membrane module, which allows the coating process to be readily and economically scaled up to a commercial level. This adsorption approach also allows for the re-application of fresh films over time during the life of the contactor. Further, the retention of the NCA as an immobilized film within the absorption contactor, avoids transfer into the desorption unit. The desorption unit is typically operated at 120-140°C [18], which would be more detrimental to the enzymatic activity.

The experiments that have been conducted in this work are short term studies (less than 1 day) that do not factor into account any enzyme deactivation kinetics, which will reduce the reaction enhancement over a longer period of time. The presence of toxic impurities such as sulfur dioxide (SO₂) and nitrogen dioxide (NO₂) in flue gases may further inhibit the enzyme activity [2]. These issues will be addressed in our ongoing work.
Chapter 6: The Robustness of Carbonic Anhydrase Enzyme for Membrane-Based Carbon Capture Applications

6.1 Chapter Perspective

Chapter 5 focused on optimizing the quantity of enzyme adsorbed onto hollow fiber membranes for maximizing their performance. In this chapter, the feasibility of using the adsorbed enzymes for CO₂ absorption operations is investigated in two ways. Firstly, the enzymes are exposed to a range of absorption temperatures in the contactor with 30 wt% K₂CO₃ over periods spanning 100 to 600 hours to determine their survivability during the absorption operation. Secondly, the adsorbed enzymes are exposed to dry gases such as sulfur dioxide (SO₂) and nitric oxide (NO). These gases can be found in post-combustion flue gas streams and are known to be CA inhibitors. In addition, these gases are able to react with the basic K₂CO₃ solvent to form sulfate (SO₄²⁻) or nitrate (NO₃⁻) ions, which may also possess inhibitory characteristics towards CA activity. Hence, challenge testing of the enzyme coated membranes in the presence of these ions is conducted.
6.2 Abstract

A microbial carbonic anhydrase (CA) was immobilized onto the surface of hollow fiber membranes to enhance the absorption rate of carbon dioxide (CO₂) into an aqueous 30 wt% potassium carbonate (K₂CO₃) solvent at pH 10-12. The performance of the immobilized enzyme was investigated over long time frames to determine the suitability of this approach for post-combustion carbon capture. The immobilized enzyme lost activity rapidly when used at 50°C and was completely deactivated within 4 days. Conversely, it was able to maintain activity for up to 80 days at 25°C. The effect of sulfur dioxide (SO₂) and nitric oxide (NO) species that are present in post-combustion flue gas streams was investigated by contacting enzyme immobilized onto flat planar membrane coupons directly with the dry gases and with solutions containing the anions associated with the gases, namely sulfate (SO₄²⁻) and nitrate (NO₃⁻). The immobilized enzyme maintained approximately 90% of its activity even in the presence of 200 mM SO₄²⁻ or NO₃⁻ and remained uninhibited in the presence of 500 ppm of the dry gases. Our findings indicate that the enzyme is not significantly affected by these toxic gases or their associated anions. Rather, the combination of high pH and ionic strength of the solvent at a high absorption temperature causes a reduction in the enzyme activity and affects the performance of the absorption process.
6.3 Introduction

Carbonic anhydrase (CA) has been researched extensively for its rapid hydration of carbon dioxide (CO$_2$) into bicarbonate (HCO$_3^-$) ions. The enzyme acts through a coordinated metal cation, usually Zn$^{2+}$, which binds a water molecule to form a hydroxide ion in the first step of the catalytic cycle. This hydroxide ion then reacts with the CO$_2$ molecule to form a HCO$_3^-$ ion, with hydration rates ranging from $10^4$-10$^6$ molecules of CO$_2$ per molecule of CA per second [2, 139, 147, 149, 156, 157]. This hydration can be beneficial for increasing the absorption efficiency of CO$_2$ into a solvent for carbon capture and storage (CCS) operations. The enhanced absorption rate is especially useful for solvents that exhibit absorption kinetics that are much slower than conventional monoethanolamine (MEA), including tertiary amines [30, 34, 69, 71] or potassium carbonate (K$_2$CO$_3$) [6].

Recent work has focused on the development of thermally stable CA variants that can withstand temperatures of up to 80°C [175, 176]. Zhang et al. [50] have also shown that a microbial CA variant obtained from Novozymes was able to maintain 60% of its activity when kept in a 0.1 M K$_2$CO$_3$-KHCO$_3$ solvent (pH 10.5) at 50°C for 30 days. However, within a carbon capture absorption unit that is operated with 30 wt% (2.8 M) K$_2$CO$_3$, the enzyme must contend with not only elevated temperatures of 30-50°C [35, 55], but also with pH levels of up to 12 (for fresh K$_2$CO$_3$) [114] and a high ionic strength environment. Enzyme stability or activity data obtained at lower pH and low ionic strength [50, 175, 176] does not necessarily translate into similar performance under these harsher industrial conditions.

In addition to the high ionic strength and pH of such carbon capture solvents, the CA must also be tolerant of the sulfur oxides (SO$_x$, which is mainly SO$_2$ with some SO$_3$) and nitric oxides (NO$_x$, typically 99% NO, with balance NO$_2$ and N$_2$O) found in post-combustion flue gas streams at concentrations of 100-300 ppm [30, 156, 201-203, 278-280]. During the absorption process, the SO$_x$ and NO$_x$ molecules will react
with the basic solvent and ultimately form sulfate (SO$_4^{2-}$) and nitrate (NO$_3^-$) ions, which are known inhibitors of CA activity under certain conditions [2, 143, 156, 208, 211]. In mammalian CAs, these anions are known to bind to a site that is near the active metal cation and displace the OH$^-$ that is required for the hydration reaction to proceed [209], which results in the formation of an inactive enzyme complex [210].

The concentrations of SO$_4^{2-}$ and NO$_3^-$ found in a typical MEA solvent post-absorption are typically at 15-25 mM and 10-25 mM, respectively [281]. Other reports indicate that monovalent ions are more likely to cause inhibition of CA than divalent ions [209, 210], while another report indicated that high SO$_4^{2-}$ concentrations of up to 1 M were conversely found to enhance the esterase activity of human CA in other circumstances [209].

The work to date on anion inactivation has mainly considered the inhibition of CA activity in the presence of nitrate and sulfate anions at pH 7-9, which does not reflect the true operating parameters of a CO$_2$ absorption process. In addition, the different CA variants that were tested in prior studies exhibited different levels of inhibition [2, 143, 156, 208, 211], which does not provide any obvious trends for determining the behavior of other CA variants. Information on the stability of immobilized CA in membrane contactors in the presence of these toxic gases is also limited.

In our previous work [282], we electrostatically immobilized thin layers of a modified thermostable CA onto the shell side surface of a hollow fiber membrane via the layer-by-layer (LbL) adsorption technique to ensure that the CA was as close to the gas-liquid interface as possible for maximizing the efficiency of the adsorbed CA [227]. A schematic of this process is shown in Figure 6-1:
Figure 6-1. The sequential LbL adsorption of polyelectrolyte layers (shown as random coils) and CA molecules (shown as ovals) onto a membrane. CO₂ is pumped through the lumen side of the hollow fibers and diffuses through the membrane into the liquid solvent, which is pumped through the shell side. The CA is immobilized onto the shell side for facilitating the CO₂ absorption reaction.

These layers were able to reduce the wetting of the microporous membranes and increase the overall mass transfer coefficient for CO₂ transport significantly. However, that work used pure solutions and gases that were free of NOₓ and SOₓ, as well as their corresponding NO₃⁻ and SO₄²⁻ ions. This paper aims to examine the effects of these gases and anions on the activity of immobilized CA at pH and ionic strengths comparable to the intended industrial application. We specifically analyze the impact of 30 wt% K₂CO₃ (2.8 M) with pH 10-12 corresponding to loadings of 0-0.15. The catalytic efficiency of the immobilized CA is also monitored over long-term operations with 30 wt% K₂CO₃ at different temperatures to determine the thermal robustness of the CA in the solvent.
6.4 Materials and Methods

6.4.1 Materials

Polyethyleneimine (PEI, 25 kDa), polystyrene sulfonate (PSS, 70 kDa) and polyallylamine hydrochloride (PAH, 10 kDa) were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Hydrochloric acid (HCl, 37%) and sulfuric acid (H2SO4, 98%) were purchased from Scharlau (Gillman, SA, Australia). Sodium nitrate (NaNO3), sodium hydroxide (NaOH), potassium sulfate (K2SO4) and trishydroxymethylaminomethane (Tris) were obtained from ChemSupply (Gillman, SA, Australia). Potassium carbonate (K2CO3, ≥ 99%) was obtained from Thasco Chemical Co. Ltd (Bangkok, Thailand) and dissolved in tap water to prepare a 30 wt% solution at pH 12. Purified water was obtained from an Elix purification system (Merck Millipore, Kilsyth, VIC, Australia) with a resistivity > 5.0 MΩ cm, while ultrapure water was obtained from a Milli-Q purification system (Merck Millipore, Kilsyth, VIC, Australia) with a resistivity of more than 18.2 MΩ cm. The gases, including carbon dioxide (CO2, 99%), nitric oxide (NO, 1000 ppm in N2), sulfur dioxide (SO2, 1000 ppm in N2) and H2S (1000 ppm in N2) were supplied by Coregas (Thomastown, VIC, Australia).

Tris buffer (50 mM, pH 7.2 or 6.4) was prepared in ultrapure water and the pH was adjusted by dropwise addition of hydrochloric acid (1 M), in line with the activity tests that were conducted in our previous work [273]. The polyelectrolyte (PEI, PSS, and PAH) solutions were all prepared in Tris buffer (pH 7.2) at a concentration of 1 mg mL⁻¹.

The CA variant used in this work was a developmental microbial CA produced by Novozymes A/S (Bagsværд, Denmark) via a fermentation and recovery process. The recovered liquid was stored at -4°C and only thawed to room temperature when required for use. This solution was diluted to 2% (v/v) with Tris buffer (pH 7.2) prior to LbL adsorption.

Flat sheet polypropylene (PP) membrane coupons were obtained from Sterlitech Corporation (Kent, WA, USA) with a pore size of 0.10
μm, a thickness of 75-110 μm and a diameter of 47 mm (information provided by the supplier). Nonporous polysulfone hollow fiber membranes that were coated with a 0.5 μm layer of polydimethoxysilane (PDMS) were supplied by Airrane Co. Ltd. (Daejeon, Korea). Hollow fiber membrane modules were prepared with 20 of these hollow fibers sealed within each membrane module (Table 3-2).

6.4.2 Layer-by-Layer Electrostatic Adsorption of Enzyme

The flat sheet PP membrane coupons were coated with polyelectrolyte solution to form a PEI/PSS/PAH/[PSS/PAH/CA]₂ film in the same manner as in our previous work [273]. Approximately 5 mL of polyelectrolyte solution was pipetted onto the membrane surface and spread evenly with a paintbrush at 23 ± 2°C to deposit a polyelectrolyte layer. These solutions were kept in contact with the membrane for 3 min before washing with excess ultrapure water for 5 min. Subsequent layers were then deposited in the same manner for fabricating a polyelectrolyte film via LbL adsorption.

Similarly, adsorbed polyelectrolytes in the form of a PEI/PSS/PAH/[PSS/PAH/CA]₂ film were coated onto the outside surface of the hollow fibers in a manner based on our previous work [282]. In this case, polyelectrolyte solutions were pumped through the shell side of the hollow fiber membrane module using a peristaltic pump at 23 ± 2°C. Each solution was kept in contact with the hollow fibers for 5 min to allow the polyelectrolytes to adsorb onto the hollow fibers, followed by a wash step with ultrapure water for 10 min.

6.4.3 Flat Sheet Membrane Experiments

The moist PP membrane coupons with adsorbed enzyme were placed in a stainless steel vessel. Other membrane coupons were dried under a stream of N₂ gas for 3 min before being placed in the vessel. The vessel was purged with N₂ at atmospheric pressure (1 bar abs) and pressurized with 1000 ppm of SO₂ or NO in N₂ to 2 bar abs for a final
SO₂ or NO concentration of 500 ppm in N₂, resulting in a final gas partial pressure of 1 bar abs. The membrane coupons were removed from the vessel periodically to determine the enzyme activity. After each removal, the vessel was purged with N₂ and re-pressurized again with 500 ppm of SO₂ or NO in N₂.

Other PP membrane coupons were immersed in Tris buffer (pH 6.4) containing NaNO₃ or K₂SO₄ at varying concentrations (50-200 mM). These solutions were kept at ambient temperature (23 ± 2°C) and the membranes were removed from the solutions periodically for testing as well after being dried under a stream of N₂ gas. In some experiments, the pH of the Tris solutions was adjusted by dropwise addition of NaOH (1 M) or HCl (1 M) to pH 2 and 12 for determining the CA activity upon exposure to extreme pH conditions. The pH of the solutions were monitored and found to remain approximately constant throughout the incubation period. The membranes were washed for 3 min with ultrapure water prior to conducting any activity tests to remove any residual HCl or NaOH on the membrane.

The enzyme activity on the flat sheet membranes was assayed according to the method used in our previous work [273] as well as by Vinoba and co-workers [48, 49]. A saturated CO₂ solution was prepared by passing a CO₂ gas stream into a bottle of ultrapure water at 23 ± 2°C for 1 hour. Approximately 40 mL of Tris (pH 6.4) was added to 100 mL of the saturated CO₂ solution before the membrane coupon was immersed into the mixture. The mixture was stirred for 1 minute followed by the removal of membrane coupon before the addition of 20 mL of CaCl₂·2H₂O solution (5.3% w/v) and 10 mL of NaOH solution (1 M) to quench the reaction and precipitate the Ca(HCO₃)₂ as CaCO₃. The precipitated CaCO₃ was then filtered, dried in an oven at 80°C overnight and weighed to determine the quantity of CaCO₃ produced.
6.4.4 Operation of the Hollow Fiber Membrane Contactors

A pilot plant gas absorption unit that was described previously [6] was utilized for these experiments. Pure CO₂ was fed to the lumen side of the enzyme-coated hollow fibers at 1.0-1.5 bar and 30 wt% K₂CO₃ solvent was pumped through the shell side of the fibers at a pressure differential of 0.1 bar above the gas pressure. The flowrate was controlled by the pump speed to achieve a solvent Reynolds number between 20 and 60. In long term experiments that lasted for up to 600 hours, spent solvent was pumped through a second PDMS hollow fiber module at elevated temperatures (70-100°C) at flow rates of 0.01-0.03 L min⁻¹ to release the excess dissolved CO₂ for solvent regeneration. After the first 10 h of absorber operation with fresh solvent at pH 12, regenerated solvent (pH 10-11) was pumped back into the absorber for the remainder of the experiment and then continuously recirculated between the absorber and the regenerator hollow fiber modules. The temperature of the regeneration operation and the solvent flow rate were controlled to maintain a consistent K₂CO₃ solvent concentration for recirculation into the absorption unit at a KHCO₃ loading of 0.1-0.15 and a pH of 10-11, while the feed solvent flow rate for the absorber was kept constant at 0.05 L min⁻¹.

Separate hollow fiber absorption experiments were conducted with 200 mM NaNO₃ and 200 mM K₂SO₄ added to fresh 30 wt% K₂CO₃ (pH 12) to determine the effect of the dissolved ions on the activity of the CA enzyme.

Samples of spent solvent exiting the contactor were analyzed for their K₂CO₃ and KHCO₃ concentrations via titration with a Metrohm–Titrando 809 (Switzerland) auto-titrator coupled with a pH probe. Solvent samples of approximately 2 mL each were diluted with 60 mL of RO water using a set volume liquid dispenser (Eppendorf, Varispencer, Germany). This mixture was then titrated with 0.4 M sulfuric acid to determine the HCO₃⁻ and the H₂CO₃ equivalence points. These bicarbonate concentrations were then used to determine the CO₂ flux.
(NCO₂) through the hollow fiber membranes based on the CO₂-K₂CO₃ reaction stoichiometry (Equation 2-2):

As reported in our prior work, for a pure CO₂ feed, the overall mass transfer coefficient (K) can be related to this CO₂ flux by Equation 2-4 [282].

\[
K = \frac{N_{CO₂} \times RT}{P}
\]  

(2-4)

where R is the gas constant, P is the feed gas pressure and T is the absolute temperature. This overall mass transfer coefficient can then be described as a function of the liquid phase mass transfer coefficient \(k_{L0}\) in the absence of any chemical reaction and the membrane mass transfer coefficient \(k_M\) as per Equation 2-22:

\[
\frac{1}{K} = \frac{1}{mE k_{L0}} + \frac{1}{k_M}
\]

(2-22)

where \(m\) and E are the Henry’s law constant and the enhancement factor contributed by the chemical reaction, respectively. As described in our previous work, the value of \(m\) can be calculated as 0.97 and \(k_M\) as \(7.5 \times 10^{-3}\) m s⁻¹ [282]. The value of \(k_{L0}\) can be obtained from a shell-side liquid phase mass transfer correlation provided by Li et al. [123] for hollow fibers with a packing density of 30% and for Reynolds numbers between 0 and 100, which is shown as Equation 6-1:

\[
Sh = (0.52 - 0.64\phi)Re^{0.36+0.36}Sc^{0.33}
\]

(6-1)

Where \(\phi\) refers to the packing density, \(Sh\) is the Sherwood number, \(Re\) is the Reynolds number and \(Sc\) is the Schmidt number. This then allows for a determination of the enhancement factor E provided by both the carbonate and the enzyme reactions.

6.5 Effect of Anions and Toxic Gases on the Activity of the Immobilized Enzyme

Initial experiments with moist flat sheet membranes indicated a dramatic loss of activity of the immobilized CA within the first 1-3 h of exposure to 1000 ppm of SO₂ or NO (Figure 6-2).
Figure 6-2. The activity of the immobilized CA on the moist membranes when exposed to 1000 ppm of NO or SO₂. The data were normalized to the activity of the fresh enzyme that was not exposed to any contaminant.

This loss of activity may have resulted at least partially from reaction of the gas with the enzyme. However, it might also be attributed to a dramatic fall in the pH of the moisture that is present within the membrane. Both SO₂ and NO are known to form strong acids when dissolved in water, with H₂SO₄ having a pKa of -3 and HNO₃ a pKa of -1.45 [283]. To separate these two effects, the membrane coupons were exposed separately as dried coupons to the acid gases; and to their respective anions (NO₃ and SO₄²⁻) within aqueous solutions of acidic, neutral and basic pH conditions, to determine the effect of the pH on the CA activity.
Figure 6-3. (a) The effect of pH on the enzyme activity when the PP membranes were immersed in 200 mM NO$_3^-$ for up to 200 h, (b) the effect of NO$_3^-$ concentration on the enzyme activity upon storage in NaNO$_3$ solutions of pH 2 for up to 200 h. The data were normalized to the activity of the fresh enzyme that was not exposed to any contaminant.

Figure 6-3a indicates that the enzyme activity of a coated flat sheet PP membrane is not significantly affected upon exposure to 500 ppm dry NO gas for up to 200 h. Similarly, the presence of 200 mM NO$_3^-$ at near neutral pH results in only a small activity loss of 10-15%. However, the pH plays a significant role in activity inhibition. An alkaline pH of 12 causes an activity decrease of approximately 20%, which is similar to that observed in our previous work in the absence of any nitrate [273]. Similarly, an acidic pH of 2 causes the activity to decrease by 30-40%. The activity decrease at this pH appears to be independent of the NO$_3^-$ concentration (Figure 6-3b). It is thus likely that the results presented for the moist membranes (Figure 6-2) represented conditions of pH < 2 developing when the limited amount of water present in these structures was exposed to the acid gases.

These results indicate that the Novozymes CA variant is more stable to the presence of NO$_3^-$ ions than a commercial bovine CA, which
was found to experience up to a 50% decrease in activity upon exposure to 200 mM of NO$_3^-$ at near neutral pH [2]. However, other researchers [143, 156] have shown that NO$_3^-$ does not significantly affect the activity of bovine or human CA. These results indicate that the pH is more responsible for changes in enzyme activity. This is consistent with other work that also shows that the enzyme activity depends upon the pH and the type of anions present at low pH (< 7), while the activity was independent of pH at higher pH values [193, 210, 212].

Similar trends were observed for the immobilized enzyme activity upon exposure to SO$_2$ and SO$_4^{2-}$, as shown in Figure 6-4. As shown in Figure 6-4, NCA is more stable at neutral to strongly alkaline pH than at strongly acidic pH.

**Figure 6-4.** (a) The effect of pH on the enzyme activity when the PP membranes were immersed in 200 mM SO$_4^{2-}$ for up to 200 h, (b) the effect of SO$_4^{2-}$ concentration on the enzyme activity upon storage in nitrate solutions of pH 2 for up to 200 h. The data were normalized to the activity of the fresh enzyme that was not exposed to any contaminant.

It can be observed that the SO$_4^{2-}$ anion does not negatively affect the CA activity at pH 6.4 within experimental error. However, as it was similarly observed for the NO$_3^-$ ions in Figure 6-2, the activity loss of the
CA is not as heavily dependent on the SO₄²⁻ concentration as it is on the environment pH. Again, this is in agreement with the work from other researchers [143, 156], though in other cases a 60% decrease in activity was observed for BCA based on the Wilbur-Anderson activity assay upon exposure to 200 mM SO₄²⁻ in 50 mM Tris buffer at pH 8 [2].

The immobilization of the enzyme onto hollow fiber PDMS membranes in a PEI/PSS/PAH/[PSS/PAH/CA]₂ film and its subsequent operation as a membrane contactor yielded CO₂ mass transfer performances as illustrated in Figure 6-5:

![Figure 6-5](image)

**Figure 6-5.** The overall CO₂ mass transfer coefficients in the presence of 200 mM anions. The data for the 30 wt% K₂CO₃ solvent were obtained from our previous work in Chapter 5 [282].

Figure 6-5 illustrates that the overall mass transfer coefficient in an enzyme coated hollow fiber module decreases by approximately 10% when 200 mM SO₄²⁻ or 200 mM NO₃⁻ is added to a fresh 30 wt% K₂CO₃ solvent (pH 12), consistent with the flat sheet results. The reaction enhancement factor decreases by approximately 8% from 6.2 to 5.8 when these anions are added. In practice, these anions will not be
present in the solvent at such high concentrations, so it is likely that the immobilized enzyme will retain most of its activity during typical flue gas carbon capture operations. A similar conclusion was reached by Zhang and co-workers [35, 50, 55] with a commercially available bovine CA enzyme.

6.6 Effect of Temperature on Long-Term Mass Transfer Operations

![Figure 6-6](image)

**Figure 6-6.** The overall mass transfer coefficient for the NCA-promoted absorption of CO₂ into 30 wt% K₂CO₃ as a function of time (a) at different temperatures over 100 h of operation, (b) at 30°C over 600 h of operation. The absorption was conducted with fresh K₂CO₃ solvent (pH 12) for 10 h before regenerated solvent was circulated into the absorber. K₀ refers to the overall initial mass transfer coefficient at the relevant temperatures.

It can be observed from Figure 6-6 that the overall mass transfer coefficient decreases exponentially as a function of time in 30 wt% potassium carbonate solutions (pH 10-11) due to enzyme deactivation, consistent with reports for bovine carbonic anhydrase [189]. At ambient temperatures (25°C), the CA is found to maintain a relatively stable mass transfer coefficient over a 100 h period (Figure 6-6). However, significant decreases in the mass transfer coefficient are observed at 35°C and 50°C.
Table 6-1 illustrates the initial overall and liquid phase mass transfer coefficients obtained at each different temperature, as well as the calculated initial enhancement factor (Equation 2-5). These enhancement factors are then monitored over time, which leads to Figure 6-7.

Table 6-1. The initial overall and liquid phase mass transfer coefficients at different temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>( K_0 \times 10^4 \text{ m s}^{-1} )</th>
<th>( k_L \times 10^5 \text{ m s}^{-1} )</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1.34</td>
<td>2.26</td>
<td>6.2</td>
</tr>
<tr>
<td>30</td>
<td>1.58</td>
<td>2.76</td>
<td>6.6</td>
</tr>
<tr>
<td>35</td>
<td>1.76</td>
<td>3.12</td>
<td>6.7</td>
</tr>
<tr>
<td>50</td>
<td>2.76</td>
<td>3.87</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Figure 6-7. The change in the enhancement factor over a period of 100 h at different temperatures.
This analysis shows that the immobilized enzyme can maintain an enhancement factor of a relatively constant 6.0-6.2 over 100 h of operation at 25°C. A similar enhancement factor was achieved under short term operations in our earlier work [282]. As the temperature increases from 25-35°C, the value of the liquid phase mass transfer coefficient \((k_{L0})\) increases due to reductions in solvent viscosity and density as well as increases in the solute diffusivity. A further temperature increase to 50°C results in a larger enhancement factor, though it can be observed that the enhancement factor at 50°C decreases rapidly because of a higher likelihood of enzyme deactivation. After 70 h of operation, the enhancement factor decreases to 1.6, which was found to be the value of the base enhancement factor in the absence of any enzyme at 65°C [6]. Therefore, it appears that the CA appears to be completely deactivated after 3 days of operation in the 50°C absorption process.

The activity of an enzyme \((a)\) is known to follow an exponential decay mechanism as a function of time \((t)\), according to Equation 2-31. Fitting such an exponential decay equation to the data in Figure 6-7 allows a prediction of the time required for the NCA to be completely deactivated (i.e. when the enhancement factor is unity).
As shown in Figure 6-8, the enzyme is predicted to survive in the 30 wt% K₂CO₃ solvent for approximately 80 days. Conversely, at 50°C, it will only be active for approximately 3 days. Although other Novozymes CA variants have been shown to withstand temperatures as high as 50-70°C with activity retentions of 50-70% [50, 176], the activity tests carried out in these works involved the incubation of the enzyme with a 1 M sodium bicarbonate solution at pH 8 [176] or a 0.1 M K₂CO₃-KHCO₃ solvent at pH 10.5 [50]. These environments are not as harsh as the present analysis, where the CA is exposed to an elevated ionic strength environment (2.8 M K₂CO₃) at pH 10-12, which leads to an accelerated enzyme deactivation rate. In contrast, Alvizo et al. [177] showed that a *Desulfovibrio vulgaris* CA strain engineered for enhanced stability retained up to 40% of its activity over an absorption period of 14 weeks when operated as a free enzyme at 50°C in a 4.2 M methyl-diethanolamine solvent.
The decay constant ($\lambda$) usually follows an Arrhenius relationship with temperature ($T$) as shown in Equation 2-32. This relationship is plotted for the decay constants determined from Figure 6-7 in Figure 6-9 and results in a predicted deactivation energy of the enzyme as 113 kJ mol$^{-1}$ (27.2 kcal mol$^{-1}$).

**Figure 6-9.** The Arrhenius dependency of the decay constant $\lambda$ on the absorption temperature.

The value of this deactivation energy is lower than the 121 – 197.5 kJ mol$^{-1}$ generally observed for other CA variants (Table 2-4), which indicates that the enzyme is significantly more thermostable. In prior work, carbonic anhydrase variants isolated from *Lactobacillus delbrueckii* [193] and the *Gossypium hirsutum* cotton plant [194] exhibited deactivation energy values of 185 kJ mol$^{-1}$ and 146 kJ mol$^{-1}$, respectively, based on the Wilbur-Anderson activity assay method [166] for CO$_2$ hydration. Kanbar and Ozdemir [189] obtained values of 120 kJ mol$^{-1}$ for free BCA and 360 kJ mol$^{-1}$ for BCA immobilized within a
polyurethane foam. However, their activity tests were conducted based on the hydrolysis of p-nitrophenyl acetate, which may not correlate well with the activity of CA in CO₂ hydration [163]. The greater thermal stability of the present enzyme reflects the inherent thermal stability of the enzyme variant employed, but may also be assisted by its immobilization on the membrane surface, which can increase the resistance to thermal denaturation [52].

These results indicate that if the present enzyme is to be used in a carbon capture operation with 30 wt% K₂CO₃ at high pH, lower absorption temperatures are preferred. The use of a K₂CO₃ solvent of lower ionic strength (such as 20 wt%) as well as a lower operational pH might also increase the viability of the enzyme.

6.7 Conclusions

The membrane-immobilized Novozymes carbonic anhydrase variant studied appears to be more thermostable relative to other CA variants. However, given the harsh operating conditions used in the carbon capture operation of this study, including both high pH and ionic strength, it is still expected to become completely deactivated after only 3 days at an operating temperature of 50°C. Operation at a lower temperature of 25°C would allow for 80 days of immobilized enzyme use. It may therefore be more useful to use such an enzyme-coated membrane contactor for gas separation processes and other purposes operating at lower temperatures, such as the separation of CO₂ from methane in biogas purification processes [176].

Contrary to other published work with carbonic anhydrase enzymes, the immobilized enzyme shows a good tolerance towards the presence of SO₂ and NO or their associated anions. The performance loss was only of the order of 10%, even though the concentration levels of these toxic gases (500 ppm) or their anions (200 mM) was much higher than what would normally be obtained from post-combustion gas streams. The further development of an enzyme that retains this
resistance towards gas impurities, but increases its thermostability under aggressive operating conditions would be desirable for the future of carbon capture operations using K₂CO₃ solvents.
Chapter 7: Conclusions and Recommendations

7.1 Concluding Remarks

The emissions of anthropogenic CO\textsubscript{2} into the atmosphere have to be reduced to limit the effects of global warming. Current CO\textsubscript{2} capture techniques involving the use of amine-based solvents may be extremely effective, but these amines face issues with energy consumption during the solvent regeneration process, toxicity and the necessity for constant replenishment.

By using an alternative solvent in the form of K\textsubscript{2}CO\textsubscript{3}, the energy requirement for solvent regeneration is reduced, though the absorption kinetics is also severely affected. This thesis has developed a technique for improving the absorption kinetics of CO\textsubscript{2} into K\textsubscript{2}CO\textsubscript{3} based on the electrostatic immobilization of CA onto a membrane surface to facilitate CO\textsubscript{2} absorption in an MGA absorber unit. The immobilization of the CA onto the membrane surface prevents the CA from entering the desorber, where enzyme denaturation occurs more rapidly at elevated temperatures. The thesis can be broadly classified into 3 parts:

1) The immobilization of CA onto flat surfaces to determine the enzyme loading per unit area of surface (Chapter 4);
2) The immobilization of CA onto hollow fibers to determine the effect of the CA on the enhancement of the CO\textsubscript{2}-K\textsubscript{2}CO\textsubscript{3} absorption reaction (Chapter 5);
3) A characterization of the enzyme resilience to elevated temperatures and the effects of toxic gases and their associated anions on the performance of the immobilized CA in a membrane contactor operation (Chapter 6).

In Chapter 4, the CA exhibited a linear increase in activity according to the number of CA layers that were immobilized onto the membrane surface, which allowed for a better understanding into the activity of multilayered CA films with regards to CO\textsubscript{2} hydration. The
preparation of CA films with immobilized MS nanoparticles further increased the CA loading on the membrane surface; however, the specific enzyme activity was reduced probably because of substrate diffusion limitations.

Chapter 5 consisted of an investigation of the LbL assembly technique for optimizing the performance of the immobilized CA films. Two different membrane materials were tested, which were microporous PP hollow fiber membranes and nonporous PDMS hollow fiber membranes. The effects of temperature were also investigated over the short term on these two types of hollow fibers. The PP membranes exhibited a better mass transfer performance than the PDMS membranes over the short term because the porosity of the PP membrane allowed for better mass transfer when the membrane pores were not fully wetted with solvent. However, the PP membrane pores will eventually become wetted and will experience a decline in their mass transfer performances, possibly decreasing to a level that is lower than the PDMS hollow fibers.

With the dual effects of membrane wetting and enzyme denaturation being able to cause a reduction in the mass transfer, Chapter 6 focused solely on enzyme deactivation by utilizing the PDMS membranes over a long period of time, as well as investigating the effects of toxic gases on the NCA that was immobilized on flat membrane coupons. It was found that the NCA maintained its mass transfer performance at temperatures of less than 30°C, while elevated temperatures coupled with the high solvent pH and ionic strength caused a marked decrease in the performance of the NCA over time. It took just over 3 days to completely deactivate the adsorbed NCA at 50°C, even though the NCA was found to be relatively thermostable with a low deactivation energy.
7.2 Recommendations for Future Work

The results from Chapter 6 indicate that more thermostable CA strains should be sourced for membrane contactor operations. These might include an extremely thermostable CA variant that was produced by Alvizo et al. [177], which was able to tolerate an absorption temperature of 50°C over a period of 14 weeks in a 4.2 M MDEA solvent. The immobilization of this CA variant could lead to even better contactor performances than the current NCA variant that was investigated. It should be noted in this work that the application of the CA in thin film layers onto PDMS hollow fiber membranes resulted in an absorption enhancement factor of 6.6 at 30°C in K$_2$CO$_3$, which is a significant increase over the use of K$_2$CO$_3$ at 65°C in the absence of CA, though the absorption performance is still much lower than in the presence of MEA [6].

While it would be ideal to operate the membrane contactors with a gas feed stream containing CO$_2$/N$_2$ as well as the toxic gas components, laboratory safety regulations and the lack of available space render such an absorption operation nearly impossible. It would be expected that the mass transfer rates would be lower when absorbing CO$_2$ from flue gases because of a lower CO$_2$ driving force and the presence of water vapor in these gases could exacerbate membrane wetting. It would be better in future to prepare membrane contactors with immobilized CA and use them in pilot plant trials at actual CO$_2$ capture sites to further determine the resilience of the enzyme when operated with a real flue gas from a power plant.

The LbL technique is nevertheless a great option for coating thin polyelectrolyte films onto membranes for functionalization purposes. Further work could also expand on the viability of LbL-assembled films onto membrane surfaces in other areas of membrane separation, such as in chiral separations, water purification, immunosensing and biocatalytic processes, especially for processes that can be operated at ambient temperature and moderate pH levels.
Chapter 8: Bibliography


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Student’s contribution (%) 70

Journal or book name Journal of Membrane Science

Volume/page numbers 514/556-565

Status [ ] Accepted and In-press [X] Published Date accepted/published 13/05/16

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- The student has approval to include the publication in their thesis from their Advisory Committee
- It is a primary publication that reports on original research conducted by the student during their enrolment
- The initial draft of the work was written by the student and any subsequent editing in response to co-authors and editors reviews was performed by the student
- The publication is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in the thesis

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PhD and MPhil students may include a primary research publication in their thesis in lieu of a chapter if:

- The student contributed greater than 50% of the content in the publication and is the "primary author", i.e. the student was responsible primarily for the planning, execution and preparation of the work for publication.
- It has been peer-reviewed and accepted for publication.
- The student has approval to include the publication in their thesis from their Advisory Committee.
- It is a primary publication that reports on original research conducted by the student during their enrolment.
- The initial draft of the work was written by the student and any subsequent editing in response to co-authors and editors reviews was performed by the student.
- The publication is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in the thesis.

Students must submit this form, along with Co-author authorisation forms completed by each co-author, when the thesis is submitted to the Thesis Examination System: https://tes.app.unimelb.edu.au/. If you are including multiple publications in your thesis you will need to complete a separate form for each publication. Further information on this policy is available at: graderesearch.unimelb.edu.au/preparing-my-thesis/thesis-with-publication

A. PUBLICATION DETAILS (to be completed by the student)

<table>
<thead>
<tr>
<th>Full title</th>
<th>In Situ Layer-by-Layer Assembled Carbonic Anhydrase-Coated Hollow Fiber Membrane Contactor for Rapid CO2 Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authors</td>
<td>J. K. J. Yong, G. W. Stevens, F. Caruso, S. E. Kentish</td>
</tr>
<tr>
<td>Student’s contribution (%)</td>
<td>70</td>
</tr>
<tr>
<td>Journal or book name</td>
<td>Journal of Membrane Science</td>
</tr>
<tr>
<td>Volume/page numbers</td>
<td>514/556-565</td>
</tr>
<tr>
<td>Status</td>
<td>□ Accepted and In press □ Published Date accepted/published 13/05/16</td>
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</tbody>
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B. STUDENT’S DECLARATION

I declare that the publication above meets the requirements to be included in the thesis

<table>
<thead>
<tr>
<th>Student’s name</th>
<th>Student’s signature</th>
<th>Date (dd/mm/yy)</th>
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</thead>
<tbody>
<tr>
<td>Joel K. J. Yong</td>
<td></td>
<td>08/07/16</td>
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</table>

C. PRINCIPAL SUPervisor’S DECLARATION

I declare that:

- the information above is accurate
- The advisory committee has met and agreed to the inclusion of this publication in the student’s thesis
- All of the co-authors of the publication have reviewed the above information and have agreed to its veracity
- 'Co-Author Authorisation' forms for each co-author are attached.

<table>
<thead>
<tr>
<th>Supervisor’s name</th>
<th>Supervisor’s signature</th>
<th>Date (dd/mm/yy)</th>
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<td>Sandra E. Kentish</td>
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