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Organic Solvent Stable Membranes for Defined Sequence Polymer Synthesis

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Abstract

As of 2021, there were 15 oligonucleotide drugs and over 100 peptide drugs on the market in the U.S., Europe, and Japan [1,2]. Chemically, these therapeutics are classified as defined sequence polymers (DSPs), with DSPs characterised by having a fully defined length and an exactly defined monomer sequence [3]. The current industrial manufacture of pharmaceutical DSPs is through solid phase synthesis (SPS). However, SPS is expensive, difficult to scale, and often requires extensive purification by chromatography [4].

In this work, a new DSP manufacturing platform was developed. This new technology was based on liquid phase reactions, which are fast, scalable, and have the potential to achieve high product purities. These reactions were coupled to organic solvent-based membrane purifications to achieve efficient, high purity, high yield separations of the DSP from the reaction debris. This formed the basis for the new platform technology: membrane enabled liquid phase DSP synthesis. The success of this new platform technology required the design of a new range of organic solvent stable membranes. These membranes were manufactured using a previously untested high molecular weight polybenzimidazole (PBI) polymer (60,000 Da), which produced membranes with improved long-term stability in organic solvents, compared to the standard grade of PBI (27,000 Da), which has been used for almost all other PBI organic solvent nanofiltration (OSN) membranes in literature. These membranes were crosslinked with dibromoxylene (DBX) and shown to be mechanically, thermally, and chemically stable at DSP manufacturing conditions, specifically in polar aprotic solvents, acids, and bases. Post crosslinking, the PBI membrane was graft modified with long polyether amines. By controlling the extent of graft modification, the rejection profile of the PBI membranes was customisable. Additionally, the graft modification imparted significant anti-fouling properties to the membranes, creating the first organic solvent stable membranes specifically targeting fouling resistance. These anti-fouling properties were shown to be highly dependent on the graft modifier properties and the solvent. Therefore, by maximising the graft modifier extension away from the membrane surface, the anti-fouling properties could be predicted and optimised for the membrane's environment.

These membranes were then used to manufacture the first pharmaceutical length oligonucleotide using membrane enabled liquid phase DSP synthesis. A single apparatus, single phase synthesiser was designed and constructed which exclusively used membrane separations for the purification of the oligonucleotide. A 20-mer oligonucleotide DSP was synthesised at 50% yield and 75.2% purity. This is comparable to the current industry standard of SPS and showed that membrane enabled liquid phase DSP synthesis has the potential to rival SPS for the manufacture of pharmaceutical length DSPs in the future.

Statement of Originality

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Adam Oxley

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Adam Oxley

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Adam Oxley

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Chapter 1: Introduction

Defined sequence polymers (DSPs) are molecules with a precisely defined number of monomers and each monomer has a precisely defined location in the sequence [16]. This exact level of monomer control results in DSPs possessing unique properties and functionality [17]. The most well-known examples of DSPs are biopolymers, found in nature, such as DNA, peptides, and proteins. However, synthetic DSPs have a wide range of applications, including drug delivery, fabrication of complex materials, information storage, and most importantly as therapeutics [5,18,19].

As of 2021, there were 15 DSP drugs based on oligonucleotides, and over 100 DSP drugs based on peptides, on the market in the U.S., Europe, and Japan [1,2]. Additionally, there is a sizable pipeline for DSP therapeutics currently in clinical trials [20]. This interest from the pharmaceutical industry is because DSP drugs offer high selectivity, efficacy, and allow for rational design within the field of drug development [21].

The current industrial manufacture of pharmaceutical DSPs is through solid phase synthesis (SPS). SPS binds one terminal of the DSP to an insoluble solid support. The monomers are then sequentially added to the unbound terminal in the order necessitated by the final sequence, and the polymer is grown one monomer at a time. The solid support enables fast and effective purification of any unreacted monomers or reaction debris, and the full synthesis can be conducted in a single apparatus. This enables the process to be fully automated and pharmaceutical length oligonucleotide DSP sequences, which often require around 80 reactions, can be manufactured within 24 hours. However, SPS has diffusion limited reactions, it is difficult to scale above ~10 kg per batch, and the single-use solid support is expensive [22–25].

Liquid phase synthesis (LPS) is an alternative to SPS. LPS binds one terminal of the DSP to a soluble support or ‘hub’ molecule, and this enables all reactions to be performed in a single liquid phase [26]. Liquid phase reactions enable efficient mixing, scalability, and the reactions are kinetically limited not diffusional limited. However, the purification of unreacted monomers and reaction debris becomes a significant challenge. Several different separation techniques have been proposed for LPS including

extraction, precipitation, and chromatography [27,28]. However, these techniques are time consuming, difficult to automate, and struggle to achieve high purities and yields.

Membrane separations are an alternative purification technique for LPS [29]. Membranes provide a cost and energy efficient technique for separating chemicals on a molecular level [30,31]. The separations are effective at scale, entirely liquid phase, and can be designed to ensure close to 100% retention of a target species. Industrially, membrane separations are well established in aqueous media, and they are often the favoured industrial technique for water desalination [32,33]. However, for DSP synthesis, the membrane separations must be performed in organic solvents. This is dictated by the chemistry which is required to manufacture DSPs [7].

Membrane separations in organic solvents are a relatively new field of interest [11]. Solvent stable membranes are starting to be applied in the petrochemical, pharmaceutical, and fine chemical industries, and this has led to the development of a number of commercially available solvent stable membranes [8]. However, these commercial membranes cannot be used for DSP synthesis. Almost all organic solvent stable commercial membranes target separations in a nanofiltration range. This range is defined by a molecular weight cut off (MWCO) between 200 Da and 1,000 Da [11], and corresponds to a pore size which is too small to manufacture most DSPs. Therefore, for membrane enabled liquid phase synthesis to be an effective platform technology for DSP manufacture, a new range of solvent stable membranes specifically targeting DSP purifications must be developed.

To develop membrane enabled liquid phase DSP synthesis, this work had two main aims. The first aim was to design and develop a new range of membranes which specifically target DSP synthesis. The second aim was to synthesise a pharmaceutically relevant length DSP, namely an oligonucleotide, using exclusively membrane separations for purification.

To understand the current state of the field, a full literature review into current DSP synthesis techniques, and the current membrane technology which is available for DSP synthesis was conducted in **Chapter 2**. Following the literature review, several critical research objectives were identified in **Chapter 3**. The first objective was the development of an organic solvent stable membrane for DSP

synthesis, which was evaluated in **Chapter 4**. This membrane was based on crosslinked (PBI), which was shown to be mechanically, thermally, and solvent stable. A graft modification technique was used to customise the rejection of the membranes, and the membrane was scaled to spiral wound modules. In **Chapter 5**, the process stability of the PBI membranes was studied and optimised. This chapter investigated the effect of the polymer molecular weight, dope composition, and crosslinking extent on the long-term performance of PBI membranes. In **Chapter 6**, the antifouling properties of the polymer grafted membranes were investigated. This chapter investigated the anti-fouling effect of different grafting agents in different solvents and identified the relationship between the solvent and the grafting agent properties to optimise the antifouling properties. In **Chapter 7**, a pharmaceutical-length oligonucleotide DSP was synthesised. This chapter developed a single apparatus DSP synthesiser, optimised a soluble hub molecule based on the membranes which were established in Chapters 4-6, and synthesised a pharmaceutical length oligonucleotide. Finally, in **Chapter 8**, the conclusions of this work were reviewed and the required future work which will enable the progression of membrane enabled liquid phase DSP synthesis to an industrial manufacturing platform was outlined.

Chapter 2: Literature Review

In this literature review, the applications of defined sequence polymers (DSPs), the current DSP manufacturing methods, and the potential future manufacturing methods are investigated. The review then focuses on one key manufacturing technology for DSPs: membrane separations in organic solvents. This review highlights the current advances in organic solvent stable membranes, along with the knowledge gap which needs to be filled to reliably manufacture DSPs.

2.1 Defined Sequence Polymers (DSPs)

A defined sequence polymer is a molecule with a fully defined primary structure. This means it has an exact chain length and a precisely ordered sequence of monomers [17,34]. Through perfect control of the monomer sequence, DSPs have the potential to self-assemble, store information, and exhibit highly specific bonding, interactions, and reactivity [35]. The most well-known and well-studied DSPs are biological, found in nature, such as deoxyribonucleic acids (DNA) and proteins, where the defined sequence of the polymers enables life to function, reproduce, and thrive [16]. The structures, synthesis, and applications of both biological and non-biological (synthetic) DSPs are an important research topic, and current research is focusing on the applications of DSPs for therapeutics [36], drug-delivery [37], materials science [34], data storage [38], anti-counterfeiting technologies [39], catalysis [40], protein folding [41], and optoelectronic devices [42].

2.1.1 Oligonucleotides

Oligonucleotides are important biological DSPs comprising of short sequences of DNA or ribonucleic acids (RNA), up to around 100 bases in length. An example oligonucleotide structure is shown in **Fig. 2.1a**. This oligonucleotide contains the four natural RNA bases, each consisting of a ribose sugar, a phosphate group, and a nitrogenous base. Synthetic oligonucleotides have many applications including PCR primers, DNA microarrays, and therapeutics [43–45].

Therapeutic oligonucleotides are a highly significant and rapidly growing area of drug development. The first oligonucleotide drug, Vitravene (Formiversen), was released by Novartis in 1998. It consisted of 21 monomers, in the defined sequence: 5' - GCG TTT GCT CTT CTT CTT GCG - 3'. Vitravene was

target side effects than small molecule drugs [50,51]. Additionally, the structure of oligonucleotide drugs can be identified based on knowledge of the primary target sequence and designed rationally, reducing the requirement for time-consuming iterative screening, which is common for small molecule drug synthesis [47].

Table 2.1 Name, listing company, indication, and type of FDA-approved oligonucleotide drugs, as of 2021, adapted from [13].

#	FDA Approval	Drug Name	Company	Indication	Type
1	1998 Withdrawn	Vitravene (Fomivirsen)	Isis Pharmaceuticals/Novartis Ophthalmics	Cytomegalovirus (CMV) Retinitis	ASO
2	2004	Macugen (Pegaptanib)	OSI Pharmaceuticals	Age-related Macular Degeneration (AMD) of the Retina	Oligonucleotide
3	2013	Kynamro (mipomersen sodium)	Kastle Therapeutics LLC	Homozygous Familial Hypercholesterolemia	ASO
4	2016	Defitelio (defibrotide sodium)	Jazz Pharmaceuticals Plc	Hepatic Veno-Occlusive Disease	Oligonucleotide, natural product
5	2016	Exondys 51 (eteplirsen)	Sarepta Therapeutics	Duchenne Muscular Dystrophy	ASO
6	2016	Spinraza (nusinersen)	Biogen	Spinal Muscular Atrophy	ASO
7	2017	Heplisav-B (hepatitis B vaccine, adjuvanted)	Dynavax Technologies	Hepatitis B	Cytidine phosphoguanosine (CpG) oligonucleotide as adjuvant
8	2018	Tegsedi (inotersen sodium)	Akcea Therapeutics	Familial Amyloid Neuropathies	ASO
9	2018	Onpattro (patisiran)	Alnylam Pharmaceuticals	Familial Amyloid Neuropathies	siRNA
10	2019	Givlaari (givosiran)	Alnylam Pharmaceuticals	Acute Hepatic Porphyria	siRNA
11	2019	Vyondys 53 (golodirsen)	Sarepta Therapeutics	Duchenne Muscular Dystrophy	ASO
12	2020	Viltepso (Viltolarsen)	Nippon Shinyaku with NCNP	Duchenne Muscular Dystrophy	ASO
13	2020	Oxlumo (Lumasiran)	Alnylam Pharma	Primary hyperoxaluria type 1	siRNA
14	2021	Amondys 45 (Casimersen)	Sarepta Therapeutics	Duchenne muscular dystrophy (DMD)	ASO
15	2021	Leqvio (inclisiran)	Novartis	lower low-density lipoprotein cholesterol	siRNA

The market value of oligonucleotide drugs is continuously increasing. In 2020, oligonucleotide therapeutics had an estimated market value of \$5.2 billion USD, and this is predicted to rise to \$26.1 billion USD by 2030 [52]. This is due to a large pipeline of oligonucleotide therapeutics in clinical trials, and the recent approval of drugs with large patient populations. Most approved oligonucleotide drugs were designed to treat rare diseases which only affect a small patient population [53]. However, Leqvio (Inclisiran) was recently approved by the FDA. Leqvio targets a large patient population, and there are many more drugs targeting large patient populations in clinical trials [54–56].

2.1.2 Peptides and Proteins

Peptides and proteins are also important biological DSPs. They are composed of amino acid building blocks, covalently joined with amide bonds, shown in **Fig. 2.1b**. There are twenty naturally occurring amino acids which consist of a carboxylic acid group, an amine group, and a side group (or R group). Peptides and proteins are used biologically for many applications including cell adhesion, structure, transport, catalysis, and reproduction. Their function comes from a defined primary monomer sequence and any mistakes in the primary sequence may affect the secondary structure and results in a loss of biological function [57–59].

Peptides are defined as a single amino acid chain with around 50 or fewer amino acid monomers. The first therapeutic peptide was insulin, purified from an animal pancreas in the 1920's. Since then, over 80 peptide drugs have been approved by the FDA, with a further 150 currently in clinical trials [60]. These drugs are used for the treatment of many disorders including cancer, metabolic diseases, and osteoporosis. The market share of peptides is increasing due to their low structural complexity and high efficacy [57,61,62]. The market for peptides in 2020 was valued at \$28.5 billion USD, with a compound annual growth rate of 9.66% [63].

Proteins are much larger than peptides and can be made up of multiple chains of amino acids in a complex 3D structure. The first protein therapeutics were antibodies used in serum therapy in the 1890's. However, the size of the protein therapeutic market didn't rapidly increase until the 1970's. Since then, the market has expanded to around 10-fold higher than the peptide market, due to the

development of recombinant protein production. and in 2018, eight of the top 10 best-selling drugs worldwide were biopharmaceuticals [64,65]. These drugs are often manufactured from recombinant methods, which will be discussed in **Section 2.2.1**. Protein drugs are wide ranging and include antibodies, anticoagulants, blood factors, bone morphogenetic proteins, engineered protein scaffolds, enzymes, fusion proteins, growth factors, hormones, interferons, interleukins, and thrombolytics [66]. One of the most important classes of protein drugs are antibodies. As of the end of 2019, 79 therapeutic monoclonal antibodies had been approved by the FDA. These were valued at a market size of \$115 billion USD [67].

2.1.3 Synthetic DSPs

Oligonucleotides and peptides are both biological DSPs. However, the utilisation of synthetic polymer backbones, side groups or functionalisation allows for the creation of novel materials with unique physiochemical, structural, biological, and mechanical properties at a molecular, polymer, and macro level [19]. An example synthetic DSP is shown **Fig. 2.1c**.

There is huge breadth in the potential applications of synthetic DSPs. At a molecular level, DSPs allow for the storage of information. By utilising a coded monomer system, information can be coded into polymer sequences in the same way as DNA holds a genetic code. This allows for vast amounts of information to be stored, with each bit of data only requiring between 0.2 and 1 nm of polymer length. This method of data storage has potential applications in long term ambient temperature data storage and anti-counterfeiting technology [17,18,68–70].

Also at a molecular level, the spatial organisation of functional groups on polymer side chains promotes unique applications. For example, in drug delivery, where a drug can be conjugated onto a polymer, with an adjacent targeting ligand, and imaging agent. This enables the drugs to have increased efficacy and improved dosing [5,71].

At a polymer scale, DSPs can exhibit predictable self-assembling structures, which can be controlled by tuning the branching, functionalisation, hydrophilicity, hydrogen bonding, and charge of the polymer. This has potential applications in catalysis, biosensing, drug delivery, and optics [40,72–75].

Finally, at a macroscale, bulk properties of the polymer can be controlled, including mechanical properties, optical properties, solution behaviour, gelation, conductivity, and degradability. Although these methods are very expensive for tuning bulk properties, some drug delivery or tissue engineering applications may require this level of fine control to ensure stability, biocompatibility, or controlled degradation inside the body [17,76,77].

2.1.4 Defined Sequence Poly(ethylene glycol)

Unlike other synthetic DSPs, defined poly(ethylene glycol) (PEG) does not have variable side groups or functionality; PEG consists of one single repeating monomer unit. Therefore, the defined sequence definition of PEG is synonymous to unimolecular PEG, or monodisperse PEG, and produces a polymer with one single molecular weight. This means it has a polydispersity index of exactly 1, shown in **Fig. 2.1d**. For most applications, exact control of the molecular weight is not essential, and a low polydispersity index is acceptable, however, for pharmaceutical applications, unimolecular PEG is predicted to have significant benefits [78,79].

The conjugation of PEG molecules to pharmaceuticals is used to improve the pharmacokinetics and pharmacodynamics of a drug [80]. The technique of PEGylating pharmaceuticals was pioneered in the late 1960s by Frank Davis at Rutgers University. The group conducted work on the PEGylation of polypeptide drugs and found that the conjugated drugs had reduced kidney clearance, higher aqueous solubility, and reduced toxicity [81]. From this initial work, first generation PEGylation was born. This involved attaching PEG to the amino group of lysine on peptide-based drugs. Although this technique was successful at conjugating the drug, the number of PEG molecules which bond to lysines along the peptide backbone is difficult to control. This resulted in isomers of different molecular weights, making batch to batch reproducibility difficult [82]. A further problem was the low purity of commercial PEG available at the time, with the conjugated methoxy-PEG contaminated with up to 15% diol [83]. Any diol present led to unwanted cross-linked conjugates and altered the drug's efficacy [84]. There are two first generation PEGylated drugs currently on the market; Adagen and Oncaspar [85].

After the success of first-generation PEGylated drugs, the second-generation began clinical trials in the 1990s. Second-generation PEGylation uses site specific binding of PEG, with molecular weights from 300 to 60,000 Da [86,87]. The site specificity of the conjugation ensures minimal loss of biological activity, while also reducing the immunogenicity of a drug, and prolonging blood residence time [80]. The combination of these two effects has resulted in a total of 14 marketed PEGylated drugs, with a further 20 in clinical trials as of 2015 [88].

Unimolecular PEG has been shown to give different properties to low dispersity (non-unimolecular) PEG. Yu et al. showed that monodisperse polidocanols (an ether of dodecyl alcohol and PEG which is the active ingredient in Asclera) have higher activity and safety than regular polydisperse polidocanols when used in cell culture studies [89]. Kinbara et al. also synthesised unimolecular PEG with specific 2D shapes and showed that the properties of the specific shapes of PEG were highly unique, with a capability to prevent protein aggregation [41].

In pharmaceuticals, removing the polydispersity of the PEG may decrease the pharmacokinetic uncertainty that polydisperse PEG integrates into drug behaviour, and improve the consistency of the residence time, toxicity, and bioaccumulation of PEGylated pharmaceuticals. Additionally, it will remove the effect of batch-to-batch variations of the PEG and generate more information about the site of PEGylation as the location of conjugation is easier to analyse with a unimolecular PEG conjugate. Therefore, unimolecular PEG is a significantly important DSP.

2.1.5 Demand for DSPs

From the review of four important classes of DSPs: oligonucleotides, peptides, synthetic DSPs, and defined sequence PEG, it has been shown that DSPs are highly important molecules which are continuously increasing in market demand. Manufacturing these DSPs efficiently, and at low cost, is essential for exploiting the benefits of new and existing DSP applications.

2.2 DSP Manufacture

DSPs can be manufactured biologically or synthetically, with synthetic DSP synthesis often divided into two subgroups: solid-phase or liquid-phase synthesis.

2.2.1 Biological Synthesis

Biological synthesis is a highly effective method of manufacturing natural DSPs, such as proteins. This process predominantly uses recombinant DNA techniques which express a protein in a host organism. Once manufactured, the protein is harvested from the host cells and purified. Biological techniques are effective, well-established, and can efficiently produce large and complicated DSPs at large scale. An additional benefit of using biological techniques for protein production is *in vivo* folding and post translational modifications of the final protein, which is difficult to achieve synthetically. However, the use of living cells makes the process susceptible to viral infections and the process involves highly complicated purification procedures [64,90,91]. Additionally, biological synthesis is largely limited to biological DSPs. Although there have been a number of recent publications working on the incorporation of non-natural monomers in biological synthesis, current practices are predominantly limited to natural DSPs, which only contain natural amino acids or natural DNA or RNA bases [92,93].

2.2.2 Synthetic Synthesis

Natural DSPs can also be manufactured synthetically. While proteins are manufactured biologically, most therapeutic peptides are manufactured synthetically, with several notable exceptions including glucagon and liraglutide [94,95]. The choice between biological or synthetic manufacture is often determined by the length of the target DSP. Synthetic manufacture is often cheaper, quicker, and highly flexible, with nonnatural monomers easily incorporated in synthetic manufacture. However, declining purities with increasing sequence lengths limit synthetic synthesis. In literature, sequences up to, and above, 100 monomers have been published. However, in industry DSPs above 30 monomers are rarely produced synthetically [96,97].

Therapeutic oligonucleotide DSPs are also manufactured synthetically. This is partly due to their small size, as most therapeutic oligonucleotides are less than 30 monomers in length [47]. Additionally, this is because oligonucleotide therapeutics typically have non-natural modifications. Backbone modifications, nucleobase modifications, 2' substitutions, or 2' bridges, improve the cellular uptake, binding affinity, and aqueous solubility of the drugs, while also reducing enzymatic degradation.

However, these modifications make it difficult to manufacture these therapeutics biologically, and therefore synthetic manufacture is currently essential for the production of therapeutic oligonucleotides [4,20,98].

Iterative Synthesis

The synthetic manufacture of DSPs cannot be achieved through conventional polymerisation techniques. DSPs possess a fully defined monomer sequence, which cannot be attained through conventional techniques. Instead, they must be manufactured iteratively. Iterative synthesis is a cyclic process, and the completion of each cycle results in the addition of one single monomer to the polymer chain.

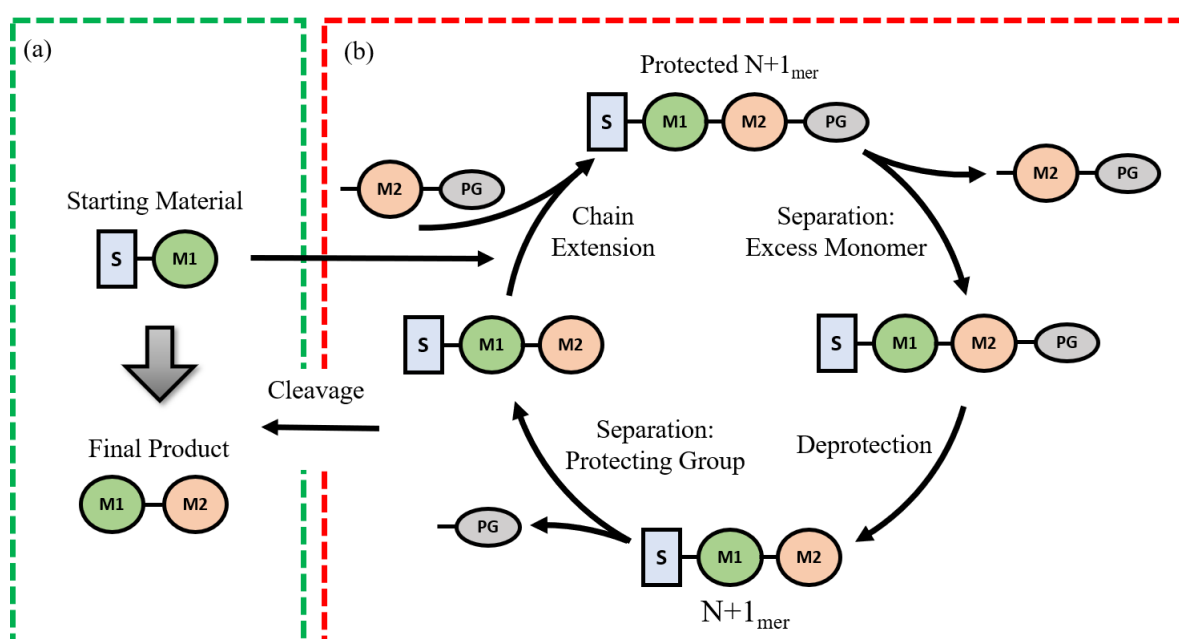


Fig. 2.2 (a) The target reaction. The starting material is the first monomer, 'M1', bound to a support, 'S'. The target final product is the DSP 'M1-M2'. (b) The iterative cycle made up of a chain extension with addition of an excess of a protected monomer 'M2-PG', separation of the excess monomer 'M2-PG', deprotection of the protecting group 'PG', and separation of the protecting group debris 'PG'. This cycle extends the polymer by a single unit. This cycle is iterated to synthesise the required defined sequence polymer and the final sequence is cleaved from the support to produce the final product.

An example of the cyclic iterative synthesis process is shown in **Fig. 2.2**. To obtain a desired DSP, the first monomer (M1) of the sequence is added to the system, often coupled to a solid or soluble support (S). This first monomer is ‘chain extended’ by reacting with a molar excess of the second monomer (M2) in the sequence to form a dimer. The reactive monomer should contain a stable protecting group (PG) to ensure only one single monomer is added. Then when the reaction is complete, all unreacted reagents, such as excess monomer or reaction debris, must be separated out of the system. Following this, deprotection of the dimer is required and, when complete, the deprotection debris are again separated out of the system. This molecule can then be chain extended again, and the cyclic process of chain extension, separation, deprotection, and separation is iteratively repeated. This builds up the desired polymer chain by adding one monomer at a time, in the order defined by the DSP sequence. Finally, once the final sequence is attained, the polymer is cleaved from the support to produce the desired DSP. This cyclic process has many variations including additional reaction steps, additional separation steps, and different supports. However, the iterative cyclic process is essential for the synthetic manufacture of DSPs [6,29,78,99].

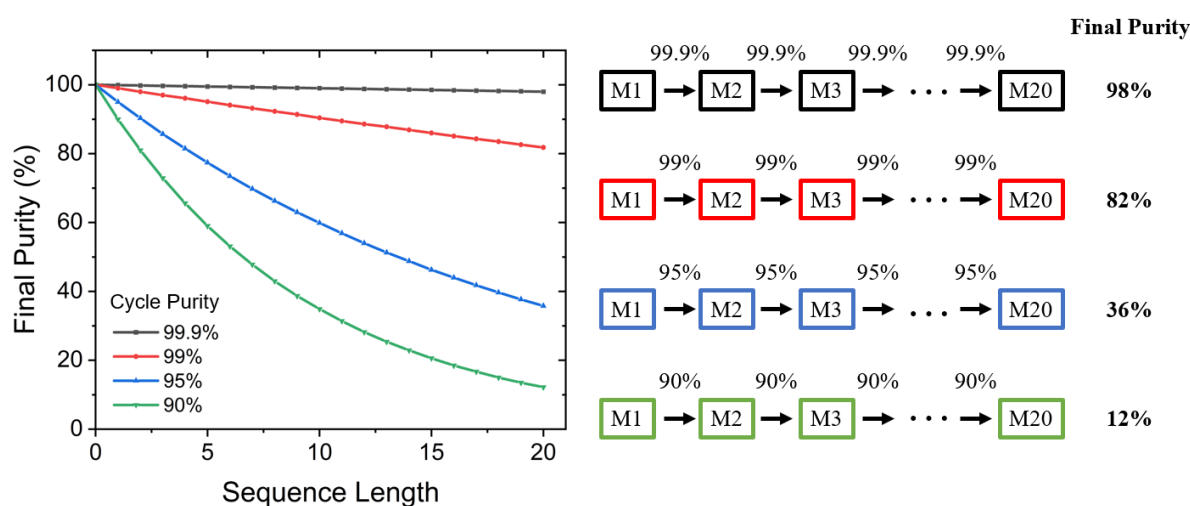


Fig. 2.3 The consequence of <100% cycle purity on the final product purity for the iterative synthesis of a 20-mer DSP with a monomer sequence M1, M2, M3...M20. The calculated final purity of 98%, 82%, 36%, and 12% is shown for 99.9%, 99%, 95%, and 90% cycle purity respectively.

Due to the iterative nature of DSP synthesis, and the resulting number of process steps, every step of the process must have close to 100% completion to achieve a high purity final product. The consequences of a reaction or a separation having low purity is shown in **Fig. 2.3**. The overall purity is equal to the purity per cycle to the power of the number of iterations per cycle. For example, if every iterative cycle has 99.9% purity, after 20 cycles the purity will be $0.999^{20} = 98\%$ overall purity.

Solid Phase Synthesis (SPS)

The standard method for industrial synthetic DSP manufacture is iterative solid phase synthesis (SPS). This is based on the iterative synthesis process shown in **Fig. 2.2**, where ‘S’ corresponds to an insoluble solid support. This technique was developed by Merrifield for peptide synthesis in 1963 and has since become the dominant industrial technique for therapeutic peptide and therapeutic oligonucleotide synthesis [22,100]. From this point in the literature review, the focus will be exclusively on oligonucleotide synthesis, specifically the industrially used phosphoramidite approach to oligonucleotide synthesis. However, the methods discussed are also applicable to other oligonucleotide synthesis techniques, peptide synthesis, and synthetic DSP synthesis.

The commonly used phosphoramidite approach for solid phase iterative oligonucleotide synthesis is shown in **Fig. 2.4**. The first step is to load an insoluble solid support, or resin, with the first monomer in the sequence, reacted onto the 3' - end to form the starting material for the process. This resin is then packed into the synthesis column and held in place between two fixed filters. After this, the cyclic synthesis process is iteratively performed comprising of four steps: activation and coupling, capping, oxidation or sulphurisation, and detritylation. Between each of these steps, the column is thoroughly washed to remove any excess reagents or reaction debris. The process is similar to the general iterative synthesis cycle shown in **Fig. 2.2**. However, the manufacture of oligonucleotides requires two additional reaction steps in the cycle, capping and oxidation or sulphurisation, and therefore two additional washing steps. One iteration of this cycle adds on one monomer, and the monomers are added in the sequence specified by the final oligonucleotide. After the final washing step, the product is cleaved from the solid support and the oligonucleotide bases are fully deprotected [94,101–103]. The

final oligonucleotide is then purified, often with anion exchange chromatography or reversed phase chromatography, and then ultrafiltered to remove any impurities [104].

The main benefit of SPS is the high efficiency of the separations. The use of a solid resin means that the separation is a simple washing process, which is completed by continuously passing fresh solvent through the fixed bed of solid resin. This process is effective and highly efficient, requiring a low volume of solvent to fully wash away all unreacted reagents and reaction debris. An additional advantage of the efficient separations is that a large excesses of every reagent can be used. This is often used to drive the reactions to completion and, because of the efficiency of the washing process, there should be no reagent present in the next reaction stage, which would result in a decrease of the final product purity [6].

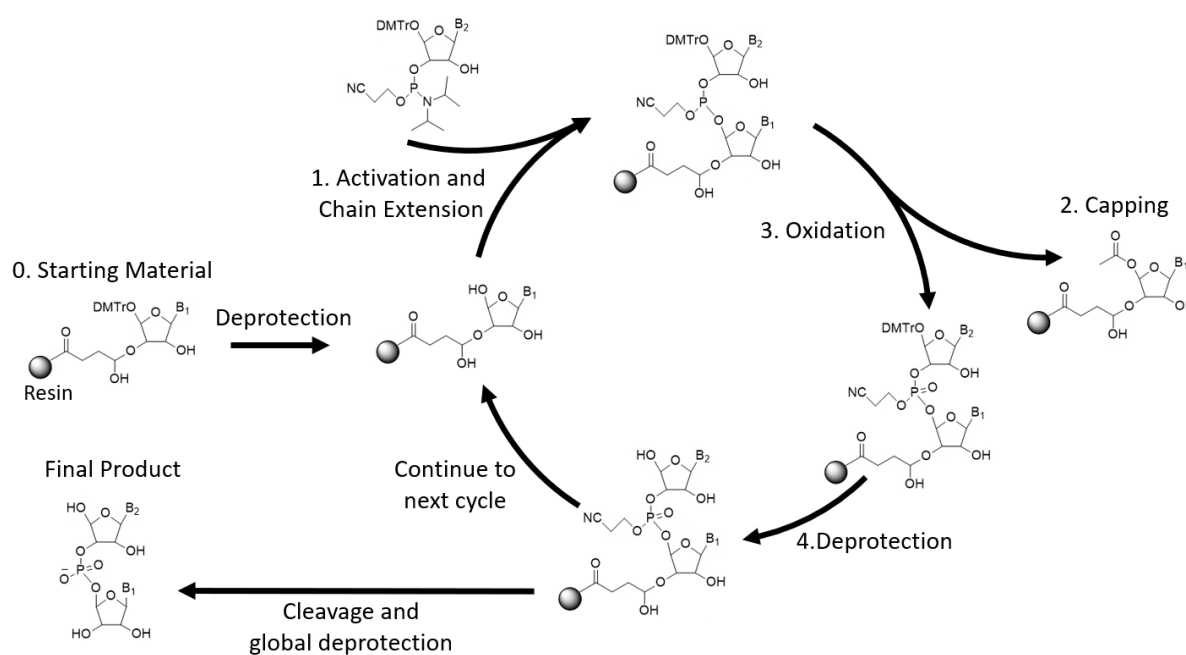


Fig. 2.4 Solid phase iterative synthesis of an RNA oligonucleotide by the phosphoramidite approach, with the chemical structures shown for the synthesis of a dimer: B1-B2. The process is cyclic, and every cycle adds one monomer. Each cycle consists of 1. Activation and chain extension, 2. Capping, 3. Oxidation or sulphurisation (oxidation is shown), 4. Deprotection. The last step after the iterative cycle has been repeated 'n' times, is cleavage and global deprotection to produce the final full-length product, where 'n' is the number of monomers in the target oligonucleotide sequence [6].

Due to the efficiency of the process, SPS is a well-established industrial process. Cytiva are one of the leading companies which manufacture equipment to make oligonucleotides by SPS. Their large-scale OligoProcess equipment can use up to twenty-four different types of monomer to synthesise up to 1.8 mol of pharmaceutical oligonucleotide per batch in a fully GMP compliant process. The efficiency of the washing, and the ability to perform the entire synthesis in a single vessel, allows for the manufacture of oligonucleotides to be fast and fully automated. This results in the manufacture of a 20-mer oligonucleotide, which requires 80 reactions, in around 8 hours processing time [105,106]. The final oligonucleotide purity is highly dependent on the sequence and the operating conditions, but after purification a high purity oligonucleotide (~90%) at moderate yields (~50%) can be achieved using SPS [4].

However, there are disadvantages to SPS. Industrial SPS binds the growing oligonucleotide to a solid resin, often porous polystyrene beads between 50 μm and 200 μm are used. During the oligonucleotide synthesis the reactants have to diffuse into the porous resin and the intraparticle reactions are significantly limited by the rate of diffusion in the resin. This often controls the rate of the chain extension reactions and can lead to incomplete reactions. Additionally, as the oligonucleotide grows inside the porous resin, the rate of diffusion is slowed by the presence of the growing oligonucleotide, further decreasing the rate of the reaction. This results in the product purity significantly decreasing as the length of the oligonucleotide increases. To compensate for this, excess reagents are used to increase the diffusion gradient and drive the reaction towards completion. However, this translates to an exponentially increasing cost of oligonucleotide manufacture as the length increases, and it does not fully compensate for the decrease in rate due to diffusional limitations [28,107].

A second disadvantage of this process is poor scalability. The solid resin swells significantly as the oligonucleotide grows. This swelling can lead to uneven flow distributions and channelling inside the packed bed. As SPS is scaled up, it becomes significantly more difficult to evenly distribute the flow of reactants. This results in a decrease of the purity of long oligonucleotides at large scale. Currently,

industrial SPS is limited to a maximum batch size of around 5-10 kg. Therefore, the manufacture of large quantities of oligonucleotides requires expensive parallelisation [25,108].

The final disadvantage of SPS is cost. The solid resins are single use and expensive, accounting for up to 40% of the final oligonucleotide cost [109]. Therefore, any process which can remove the resin from oligonucleotide manufacture will result in significant cost savings [101].

Liquid Phase Synthesis

Liquid phase iterative synthesis has been proposed as an alternative method of DSP synthesis. Liquid phase synthesis (LPS) is very similar to SPS. The process involves the exact same cyclic series of reactions, shown in **Fig. 2.4**. However, while solid phase synthesis anchors the DSP on an insoluble support or resin, liquid phase iterative synthesis anchors the DSP on a soluble molecule or protecting group, known as a 'hub'. All reactions are then performed entirely in the liquid phase and the washing step is replaced by a different separation technique which can purify the soluble hub molecule from the excess reagents and reaction debris.

The main advantage of LPS is homogenous reactions. Because the reactions are entirely in the liquid phase, the reactions are usually rate limited rather than diffusional limited or mixing limited. This often achieves higher reaction conversions and requires a lower excess of expensive reactants [4]. Additionally, the purity of the DSP does not decline significantly with length enabling the synthesis of long oligonucleotide sequences at high purity. Also, LPS does not use a solid support which can cause uneven distribution of reactants. Therefore, LPS is scalable, with the potential to manufacture tonnes of DSP per batch. This means LPS should benefit from economies of scale and, coupled to the removal of the expensive solid phase resin from the process, means that LPS should be cheaper than SPS [107,110,111].

However, the main challenge of LPS is trying to achieve efficient and high yield purifications. Various techniques have been evaluated for LPS including extraction, precipitation, chromatography, and membrane separations. However, these separation techniques are slower and not as effective as purification by washing in SPS.

Precipitation is a potential separation technique for LPS. Oligonucleotides have been synthesised on soluble PEG hubs and purified by precipitation in diethyl ether. The PEG hub had an added advantage of increasing the oligonucleotide solubility in the reactions. Using this technique, oligonucleotides up to 20 monomer units in length (20-mer) were synthesised, and most recently, a 15-mer was synthesised on a 10 kDa mPEG to moderate overall yield (58%) and moderate purity (66%) [28,112–114]. Other attempts to use precipitation focused on using long soluble alkyl chains to synthesise 21-mer using the Ajiphase process [26], a soluble ionic liquid: imidazolium tetrafluoroborate to improve the loading capacity in the synthesis of a tetramer [109], and pentaerythritol-derived cores to synthesise a pentamer at 43% yield [115].

Extraction has also been used to purify oligonucleotides in LPS, with hubs based on adamantyl tags to facilitate efficient separations. However, only 67% crude yield and 39% overall yield was obtained for a hexamer [111]. Flash chromatography with acetylated and methylated β -cyclodextrin hubs was also tested, however chromatography is difficult to achieve very high yields and scale [116]. Finally, fluorine tagged oligonucleotides have been used to facilitate efficient separation of failed sequences using precipitation [117].

While not strictly liquid phase, a number of studies have also investigated combining the advantages of liquid and solid phase synthesis by using solid supported reagents. However, these techniques have only been used at small scale to make short sequences [101,118,119]. Alternatively, product anchored sequential synthesis (PASS) was developed to try to combine the benefits of solid and liquid phase synthesis by using liquid phase reactions, followed by attachment to a solid support to facilitate solid phase separations. An 11-mer oligonucleotide was synthesised at 72% purity but only 15% yield, and one of the main problems was loss of the 2-cyanoethanol protecting group in the synthesis [120].

However, despite recent advances in liquid phase synthesis, these technologies are yet to rival solid phase synthesis. The main reason is the difficulty achieving 100% impurity removal and 100% product retention in the purification. Without 100% retention of the product, the yield quickly decreases as the sequence length increases. Additionally, for extraction or precipitation, multi-step purifications are

often needed, which are labour intensive and challenging to scale up. Also, the separations are a function of the properties of the DSP, which are dependent on both the length and the sequence being synthesised. Therefore, the separation conditions need to be optimised at every stage in the process. Finally, these techniques often require material transfer between apparatus. This produces a lack of process continuity and prevents the construction of a fully automated synthesiser, similar to the current industry standard in SPS. This makes LPS time consuming and increases the chance of errors in the sequence.

Membrane Enabled LPS

Due to the disadvantages of extraction, precipitation, and chromatography based iterative synthesis, membrane enabled DSP synthesis has been proposed [108]. Membranes have the potential to achieve quantitative separation yields and are highly adaptable to both scale up and scale down. Additionally, membrane enabled synthesis is the only DSP synthesis technique which features only one single phase. This enables the synthesis to be performed in a single apparatus, which means that the growing oligonucleotide never has to be transferred to a separate vessel for the separation. This is similar to SPS and makes it possible to manufacture a fully automated synthesiser, an important condition for the successful industrialisation of LPS. An example of membrane enabled LPS is shown in **Fig. 2.5**.

Membrane purifications in LPS can be performed in aqueous or organic solvents [11]. However, the DSP synthesis reactions are water sensitive and must be conducted in organic solvents [6]. Therefore, the use of aqueous-based membrane separations would necessitate full evaporation of the organic reaction phase and redissolution into an aqueous phase for every separation step. This is time consuming, has a high energy requirement, and the evaporation temperature may degrade the DSP. Therefore, membrane separations in organic solvent are essential for the efficiency and success of membrane-enabled DSP synthesis.

Due to the potential scalability, high purities, and high yields, membrane enabled liquid phase DSP synthesis in organic solvents will be the focus of this work. This process has been reported in literature for the synthesis of a number of specific polymers including defined sequence polyethers [5], peptides

[99], and oligonucleotides [7,29]. These studies are summarised in detail in **Section 7.1**, and they prove the concept of membrane enabled LPS. However, the previous examples of membrane enabled DSP synthesis had a number of limitations including a large number of material transfer steps, complex multi-stage separations, and low yields. A significant number of the difficulties were due to poor membrane separations, and it was established in the previous work that the critical part of this technology is the ‘right’ membrane [7,29,99,121].

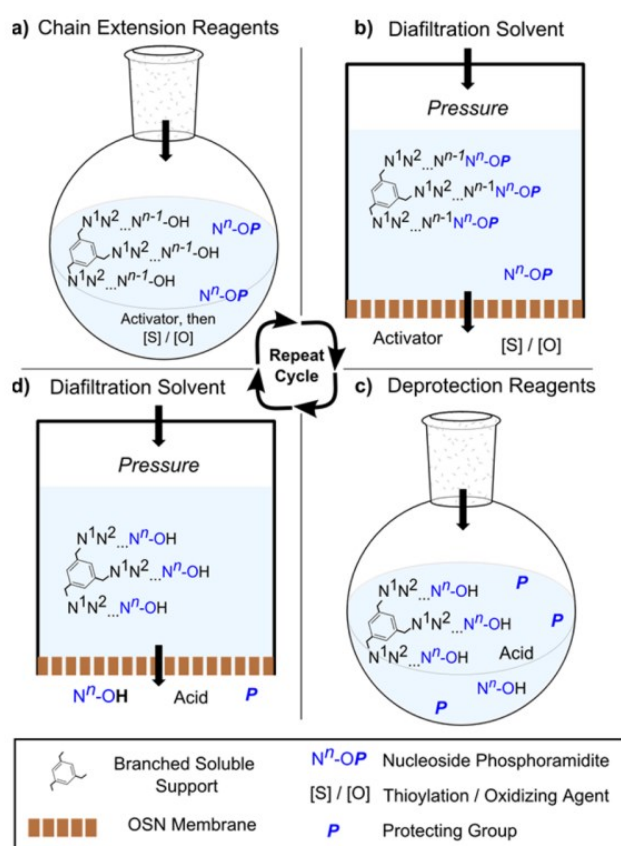


Fig. 2.5 Liquid phase synthesis of RNA on a homostar support through membrane separation. Reproduced with permission from [7].

2.3 Membranes: Definition and Applications

A membrane is a semi-permeable barrier between two phases, which separates an incoming feed stream into a retentate stream and a permeate stream. The permeate stream contains the molecules which have passed through the membrane, resulting in a stream with an increased concentration of the molecules with high permeability. The retentate stream contains the molecules which have not passed through the

membrane, resulting in a stream with an increased concentration of the molecules with low permeability. To enable the permeation of any chemical species, membranes require a driving force. This can be in terms of pressure, concentration, electrochemical potential, or temperature gradient [122]. An example membrane separation is shown in **Fig. 2.6**.

2.3.1 Global Potential of Membranes

Membranes perform separations on a molecular level without a phase change. This significantly reduces the energy requirement, reduces the operating cost, and improves the environmental impact of a separation [11]. In the US, chemical separations make up around 10-15% of the national energy consumption, with 49% of that energy consumption used for distillation. The use of membranes instead of distillation could reduce this energy consumption by up to 90% [123]. Additionally, membranes have a low footprint, and they are flexible; highly effective in both scale up and scale down operation. This has resulted in the application of membranes in place of many unit separations including distillation, extraction, crystallisation, and chromatography, and they are often used for water desalination [11].

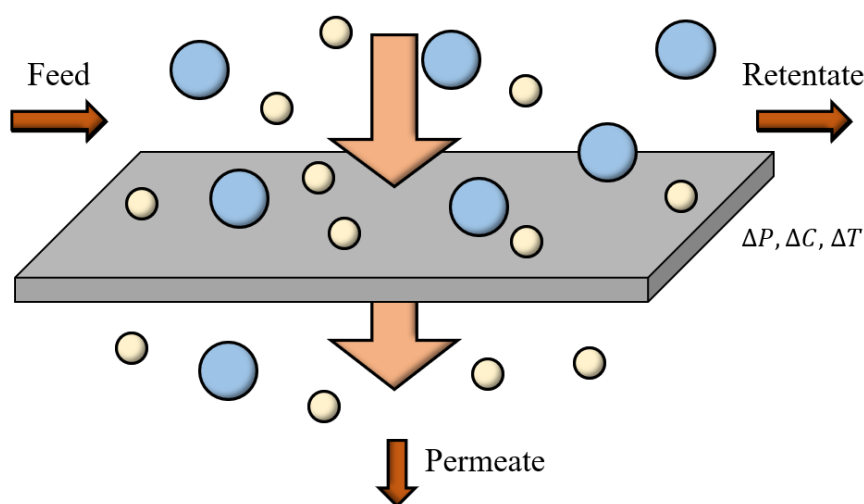


Fig. 2.6 An example of a membrane separation, separating a feed stream into a permeate and retentate stream using a pressure, concentration, or temperature-based driving force.

2.3.2 Membranes for Desalination

Membrane separations are the industrially dominant method for water purification and desalination. The current global water desalination capacity is over 100 million m³ day⁻¹, with membrane separations accounting for 65 - 80% of the global capacity. The largest global membrane-based water desalination plants purify over 900,000 m³ seawater per plant per day at a cost of around \$1.1 per cubic meter of water [32,33].

Water purification uses a series of membranes with different pore sizes, often with a pressure-based driving force. The applied transmembrane pressure is often linked to the membrane pore size, with smaller pores requiring a higher pressure to drive the liquid through the membrane. This has led to the conventional classification system for pressure-based membrane separations, which is based on the applied transmembrane pressure and approximate pore size. This classification is shown in **Table 2.2**. The required pressure increases from microfiltration, to ultrafiltration, to nanofiltration, to reverse osmosis, and the corresponding pore size decreases. For water purification, microfiltration membranes are typically used to separate bacteria, ultrafiltration for proteins and viruses, nanofiltration for sugars and divalent ions and reverse osmosis for monovalent ions. For desalination, the membranes are often used in a ‘concentration’ configuration (one solute, one solvent), but liquid phase membrane separations can also be used for solvent exchange (one solute, two solvents), and purification (two or more solutes, one solvent), shown in **Fig. 2.7**.

Table 2.2 Classification of membrane-based separations by pore size, from [14].

Classification	Transmembrane Pressure (Bar)	Approximate Pore Size (nm)
Reverse Osmosis (RO)	10 - 150	0.1 – 1
Nanofiltration (NF)	5 – 10	1 – 10
Ultrafiltration (UF)	1 – 10	10 – 100
Microfiltration (MF)	0.2 - 5	100 – 10,000

2.4 Organic Solvent Separations

While water purification is the largest global application of membrane separations, the majority of liquid phase chemical separations take place in organic solvents, including the proposed research topic of membrane enabled DSP synthesis. The application of membranes to organic solvent separations requires the specific design of solvent stable membranes. This forms the field of organic solvent nanofiltration (OSN), also known as solvent resistant nanofiltration (SRNF), or organophilic nanofiltration (ONF) [8,11,31,124].

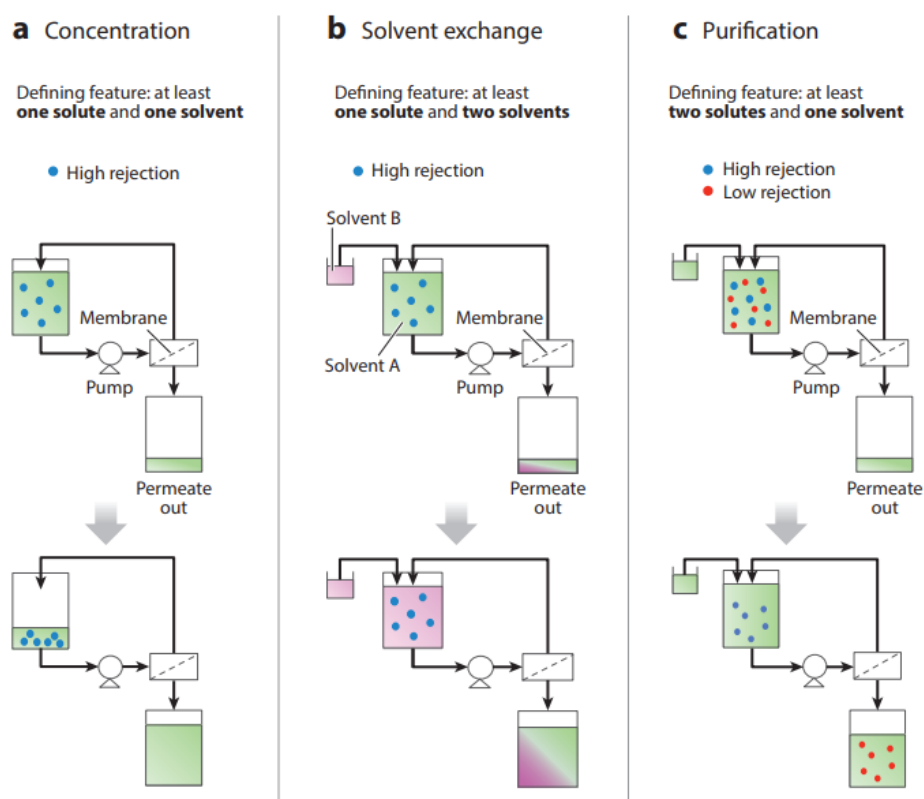


Fig. 2.7 The three applications of liquid phase membrane separations, (a) concentration, (b) solvent exchange, (c) purification. Reproduced with permission from [8].

2.4.1 Membrane Performance Indicators

To determine the applicability of a membrane for a particular organic solvent-based separation, a number of key performance indicators need to be investigated. These are often tested at lab scale, and for lab scale testing there are three main performance indicators: stability, flux, and rejection.

Stability

The most important quality of a membrane for OSN is chemical stability. The membrane must be stable in the process solvent and have consistent performance over the desired membrane lifetime. For OSN, the membrane may have to be stable in polar solvents, non-polar solvents, acidic, and basic conditions.

Stability is often tested by soaking the membrane in the process solvent and measuring the weight loss of the membrane over time. If there is no weight change, the membrane is deemed solvent stable and will be tested at lab scale for flux and selectivity [8].

Flux

Flux is the amount of liquid passing through the membrane per unit area, per unit time ($\text{L m}^{-2} \text{h}^{-1}$). The flux is often measured at the desired operating conditions of the membrane over a prolonged period of time to test the process stability of the membrane. To compare between different operating conditions, the flux is often normalised against the transmembrane pressure to calculate the permeance ($\text{L m}^{-2} \text{h}^{-1} \text{bar}^{-1}$, or LMHBar) [11]. A high permeance increases the output of a process. However, high permeances often have diminishing benefits at process conditions, as a high permeance can cause concentration polarisation and increase the susceptibility of a membrane to fouling [125].

Rejection

The third important performance indicator for OSN is the rejection. The rejection of a membrane represents the quantity of a species which can permeate the membrane relevant to the quantity retained. Rejection (R) of species 'i' is defined by equation (2.1), where C_P and C_F are the concentrations in the permeate and feed respectively.

$$R_i = 1 - \frac{C_{P_i}}{C_{F_i}} \quad (2.1)$$

The rejection is highly dependent on membrane – solute – solvent interactions and changing to a different solvent or solute will change the rejection. The rejection is also dependant on operational factors such as pressure, temperature, concentration, mass transfer, and preconditioning [9].

Most membranes are defined by a molecular weight cut off (MWCO), which is the molecular weight of the smallest solute which will have a 90% rejection by the membrane. In order to determine this, a series of compounds with known molecular weight/size are separated and the rejection of each compound is measured at dilute conditions. This generates a rejection curve and the MWCO can be determined. The MWCO is very useful for predicting the performance of concentration or solvent exchange processes, with only one solute.

However, for a purification process with two solutes, the rejection of both molecules must be known. Then, in order to select the right membrane for a purification, a separation factor or selectivity (α) can be used, defined by equation 2.2, where R_a and R_b represent the rejection of species 'a' and 'b' respectively. This defines the ability to perform the separation but not the efficiency of the process. If the selectivity is poor, multistage cascade systems can be employed to improve the separation performance [126,127].

$$\alpha_{a,b} = \frac{1-R_a}{1-R_b} \quad (2.2)$$

If the stability, flux, and selectivity are good enough for a process, then the system will be tested at pilot scale. This is performed with the configuration, hydrodynamics, and fouling conditions of the real system, to check the applicability of membranes to perform the separation of interest [128,129].

2.4.2 Membrane selectivity for DSP synthesis

DSP synthesis requires the purification of the hub from the reaction debris. Using membranes for this purification requires the hub to be retained and the reaction debris to permeate through the membrane. Therefore, for an ideal separation the rejection of the retained molecule should be ~100%, and the rejection of the permeating molecule should be ~0%. To achieve this, an ideal hypothesised rejection curve is often presented as a vertical line at a specific solute size, shown by the black dashed curve in **Fig. 2.8**. However, this idealised curve has never been shown experimentally, and many experts believe that it is not possible to achieve [130]. Sigmoidal curves are a more accurate representation of experimental membrane rejection curves, shown by the coloured curves in **Fig. 2.8**. Here, the coloured

curves are the modelled rejection profiles representing membranes with increasing pore size, where the pore sizes are in the order blue < red < yellow < purple < green [8,131].

For the purification of a DSP, the molecule to be retained is the soluble hub, which should have 100% rejection. This has been arbitrarily designated at 1.25 nm and is shown in blue in **Fig. 2.8**. The molecule to be permeated are the reaction debris and excess reagents, which should have 0% rejection. This has arbitrarily designated at 0.5 nm and is shown in red in **Fig. 2.8**.

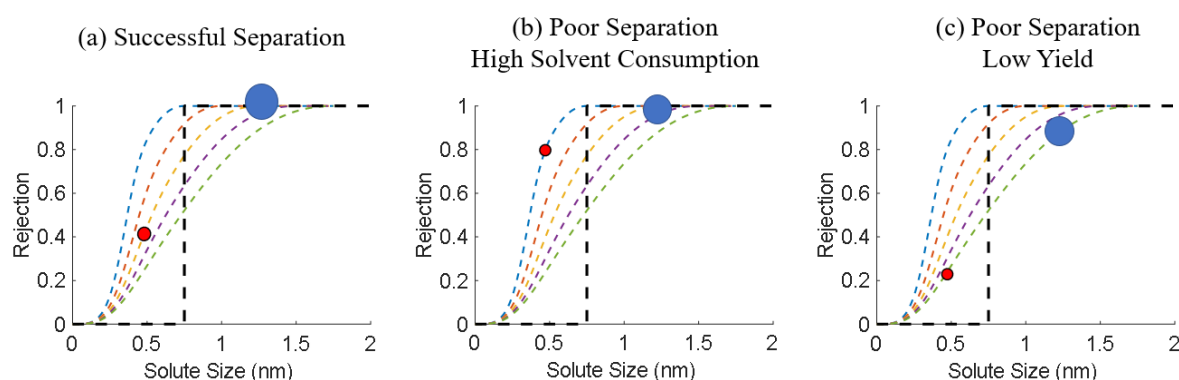


Fig. 2.8 Modelled molecular weight cut off curves for OSN with the target retained molecule shown in blue and the target permeating molecule shown in red. (a) Successful separation with high rejection of the product and low rejection of the impurities using a membrane with a 1.1 nm pore size, shown in yellow. (b) Poor separation with high rejection of the product and high rejection of the impurities using a membrane with a 0.7 nm pore size, shown in blue. (c) Poor separation with low rejection of the product but low rejection of the impurities using a membrane with a 1.5 nm pore size, shown in green. Adapted from [8].

Fig. 2.8a shows a rejection curve with the optimum membrane for DSP synthesis, shown in yellow. Most importantly, the rejection of the hub is 100%, ensuring that 100% yield will be achieved. Additionally, there is a low rejection of the monomer, 40%. Assuming that the rejection is independent of concentration, this removes 99% of the monomer in 8 system volumes, with no loss of the hub. The number of system volumes required to remove the monomer can be calculated using the differential equations for diafiltration with a constant rejection [132]. Alternatively, a tighter membrane can be used for DSP synthesis, shown in blue in **Fig. 2.8b**. The rejection of the hub is still optimum (100%).

However, the rejection of the monomer is much higher (80%). This requires 23 system volumes to remove 99% of the monomer, which is cost, time, and environmentally wasteful. On the other hand, a looser membrane can be used for DSP synthesis, shown in green in **Fig. 2.8c**. The rejection of the monomer is lower (20%), and this means that only 6 system volumes are required to remove 99% of the monomer. However, the use of a looser membrane, results in loss of the hub. The low hub rejection (90%) leads to 45% of the hub permeating during every purification which results in an uneconomical process [8].

Therefore, optimising the rejection curve specifically for the DSP synthesis is highly important. The first priority of the membrane is high membrane stability, and the second priority is a high retention of the product (>99%). After this, low rejection of the monomer and high permeance are preferred in order to minimise solvent consumption and separation time. This means that fine control of the rejection curve is essential to tune the membrane to the desired species which need to be separated.

2.5 Commercial Membranes for DSP synthesis

To select the right membrane for DSP synthesis, commercially available membranes should be considered first. A wide range of aqueous polymeric membranes are available, and they have some success in organic solvents. However, the interaction of the membrane with the solvent often leads to swelling, defect formation, or complete dissolution of the membrane. Because of this, commercial organic solvent stable membranes have been developed. These are often based on solvent stable polymers or use additional reaction steps to improve the stability of the membrane.

The first commercial polymeric OSN membranes were manufactured by Koch. They synthesised the MPF range of membranes from a polydimethylsiloxane (PDMS) layer on a polyacrylonitrile (PAN) support in the 1990s, but later discontinued this range. After Koch, W.R. Grace manufactured the Starmem series, but this range was also discontinued. Following these, a number of other companies started OSN membrane manufacture including SolSep (a wide range including many Silicon-based TFCs), Borsig (PDMS) and Evonik MET (Polyimide (PI) and Silicon-based). Recently, there has been an increase in the number of companies manufacturing OSN membranes. These include A-membranes

(ceramic membranes), Seppure (polybenzimidazole (PBI)), and Novamem (polyether ether ketone (PEEK) and polyvinylidene fluoride (PVDF)) [11,133]. These membranes are summarised in **Table 2.3**.

From the wide range of commercially available organic solvent stable membranes, none are optimal for DSP synthesis. Most commercial membranes target OSN separations ($MWCO < 1000 \text{ g mol}^{-1}$). However, DSP synthesis requires a membrane which can permeate the unreacted monomers, in the range of 300 g mol^{-1} to $2,000 \text{ g mol}^{-1}$. A common approach in membrane separations is to use a membrane with a MWCO around six times higher than the permeating species, corresponding to a wide range from 1,800 to 12,000 g mol^{-1} [134]. Therefore, a new range of membranes which target a looser MWCO must be designed to synthesise DSPs using membrane separations in organic solvents. These membranes will be defined as Organic Solvent Ultrafiltration (OSU) membranes.

Table 2.3 A range of commercial OSN and OSU membranes, adapted from [15].

Manufacturer	Membrane Series	Material	Comments
Koch	MPF	Silicon TFC on PAN	First commercial OSN membranes, now discontinued
W. R. Grace SolSep	Starmem SolSep UF and NF	PI	Discontinued
Evonik	Puramem	Diverse including Silicon TFC	Wide range of pore sizes available.
		Silicon TFC	Evonik previously made a Duramem range of crosslinked PI which is now discontinued. Widely used industrially
Borsig GMT	oNF	Silicon TFC on PAN	Widely used industrially
Novamem	PEEK, PVDF	PEEK and PVDF	5, 20 nm PEEK and 5, 20, 100 nm PVDF available
Seppure	Unknown	PBI	New company in OSN
A Membranes	Unknown	Ceramic	New company which makes custom membranes. Spin off from VITO

2.6 Membrane Development for DSP synthesis

Because there are no commercially available membranes which are suitable for DSP synthesis, new OSU membranes must be developed. Industrial applications of OSU membranes include paint recovery, dewaxing, degumming, heavy oil upgrading, and catalyst recovery [5,99,135–137]. However, these

separations have not been extensively studied using membranes and there are limited examples of OSU membranes in research. Therefore, an evaluation of the current literature on OSN membranes will be used to facilitate development of a tight OSU membrane for DSP synthesis.

2.6.1 Types of Organic Solvent Stable Membranes

Organic solvent stable membranes are typically divided into two subdivisions based on their material of manufacture: organic (polymeric) and inorganic (ceramic), along with hybrids of the two types.

Inorganic membranes

Inorganic (ceramic) membranes can be made of different inorganic materials such as Al_2O_3 , TiO_2 , ZrO_2 , SiO_2 , and they are often made through a combination of dip coating, extrusion, and sol-gel processes [138]. They have excellent chemical stability, making them ideal for OSN. Additionally, they have excellent mechanical and thermal stability, with long operational lifetimes [139–141]. Although they have been used for a number of OSN applications, their uptake is low. This is because they are often expensive, brittle, hard to produce in large quantities, and hard to make into high surface area:volume ratio modules [142]. They are also very hydrophilic and often require surface functionalisation with organic ligands to increase their hydrophobicity and make them more suitable for OSN [143].

Organic (Polymeric) Membranes

Polymeric membranes are often favoured over ceramic membranes. Polymeric membranes are cheaper and easier to manufacture than inorganic membranes, as they can be formed from solution phase. They are also much more versatile and can be made into spiral wound modules with a high surface area:volume ratio. This has led to them having much more widespread applications in industry [31,142]. However, depending on the polymer type and method of manufacture, they can have shorter lifetimes, lower chemical resistance, and increased susceptibility to mechanical and thermal damage.

There are two main types of polymeric membranes: integrally skinned asymmetric (ISA) membranes and thin-film composite (TFC) membranes, shown in **Fig 2.10** a and b respectively. TFC membranes consist of a thin highly selective layer, down to sub 10 nm, supported by a chemically stable porous sublayer [144]. They are formed through interfacial polymerization or coating techniques, including

spin coating, dip coating, and spray coating. TFCs allow for the chemistry and properties of the selective layer to be optimised independently from the chemistry and properties of the supporting layer, which provides the mechanical support [145–147].

ISA membranes consist of a dense selective layer on top of a porous support of the same material. They are normally prepared by phase inversion, and the selective layer and the sublayer form simultaneously in the phase inversion process. The selective layer is often hundreds of nanometres thick and controls the selectivity and permeance of the membrane. Although they often have lower permeance than TFC membranes, their ease of fabrication and scalability of defect-free manufacture makes them favourable for large scale OSU membranes [8,148,149].

ISA membranes for OSN are based on a wide variety of different polymers including polyimide (PI) [150,151], polyetherimide (PEI) [152], polytetrafluoroethylene (PTFE) [153], polybenzimidazole (PBI) [12,154], polydimethylsiloxane (PDMS) [155], polyvinylidene fluoride (PVDF) [156], polyether ether ketone (PEEK) [157,158], and polyacrylonitrile (PAN) [159], as shown in **Fig. 2.9**. These polymers are often selected due to their inherent solvent stability.

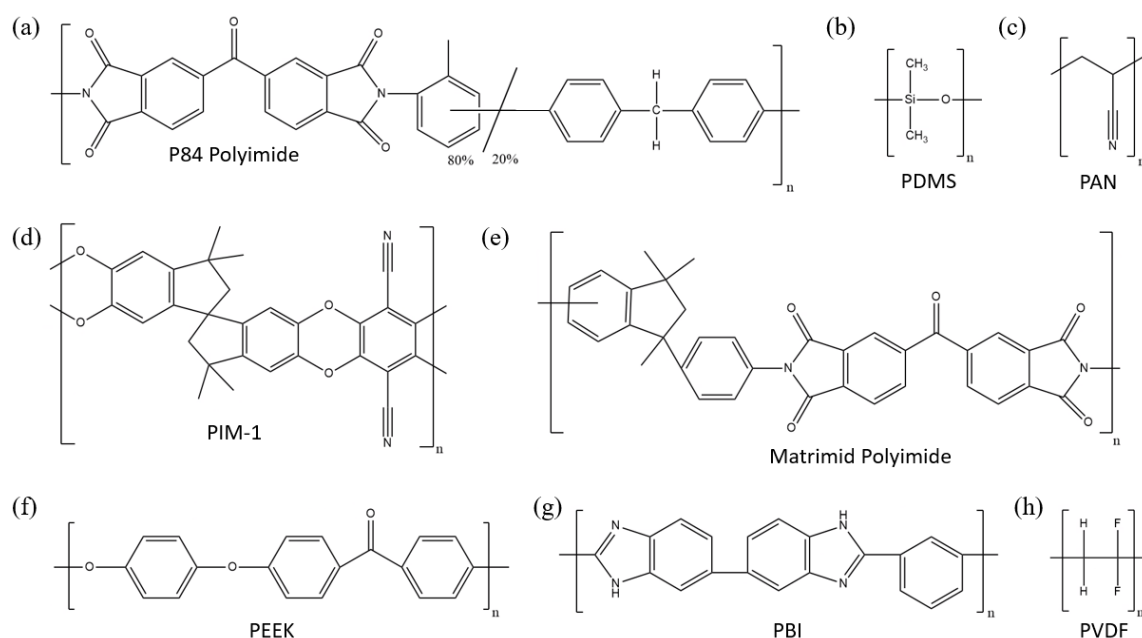


Fig. 2.9 Common polymer types used for organic solvent stable membranes (a) P84 PI, (b) PDMS, (c) PAN, (d) PIM-1, (e) Matrimid PI, (f) PEEK, (g) PBI, (h) PVDF.

Mixed Matrix Membranes

Hybrids of polymeric membranes and inorganic membranes are referred to as mixed matrix membranes (MMM). These membranes aim to combine the advantages of organic and inorganic membranes, without the disadvantages. Organic ligands between dispersed inorganic particles are often used, such as titanium(IV) oxide nanospheres [160], metal organic frameworks (MOFs) [161], covalent organic frameworks (COFs), and zeolites [162]. The combination of organic-inorganic matrix has provided membranes with increased fouling resistance, high permeance, high selectivity, and defect-free structures. Therefore, they are promising for OSN.

However, the main challenge for MMM is the elimination of grain boundaries between the organic and inorganic components. These grain boundaries are weaknesses in the membrane structure and can lead to defects. Their removal is difficult and requires improving the compatibility of the two materials and improving the membrane formation techniques [163].

2.6.2 Phase Inversion

Due to the prevalence across aqueous ultrafiltration and scalability, ISA membranes will be focused on for DSP purification. ISA ultrafiltration membranes are commonly manufactured by phase inversion. Phase inversion was developed in the 1960s by Loeb and Sourirajan and is a mature, simple, and low-cost process [149,164,165]. The first step of the phase inversion process is to make a polymer dope solution. This requires a polymer to be fully dissolved in a single solvent or a solvent mixture. This is usually in the range of 10 to 30 wt% of polymer in an aprotic solvent such as dimethyl formamide (DMF), diethyl acetamide (DMAc) or tetrahydrofuran (THF) [166]. The dope solution is then cast as a thin film and phase inverted in a controlled manner using one of the following techniques [133,152,167]:

- Immersion Precipitation – The cast membrane is immersed in a non-solvent bath. Absorption of non-solvent from the liquid phase causes precipitation of the membrane.
- Vapour Phase Precipitation – The membrane is cast in a non-solvent atmosphere. The non-solvent absorbs into the membrane from the gas phase causing precipitation of the membrane.

- Thermal Precipitation – The dope is cast at a high temperature and precipitates as it cools.
- Controlled Evaporation – The dope solution often comprises of a mixture of solvent and nonsolvent. The evaporation of solvent from a cast membrane leads to precipitation in the remaining nonsolvent.

Immersion precipitation is the most widely used phase inversion technique. This is because it has simple requirements: a polymer which is soluble in a solvent and insoluble in a different solvent (nonsolvent), and because the process is consistent and scalable [148,168]. Upon immersion in the non-solvent bath, the top layer of the membrane experiences fast phase inversion due to the high concentration of non-solvent present. This causes formation of a dense selective top layer. This top layer then slows the rate of nonsolvent diffusion into the bulk of the dope solution. Slowing the rate of non-solvent diffusion causes slower phase inversion. This creates a highly porous macrovoid structure in the polymer matrix, which has low resistance to permeating solvent and solutes [152], shown in **Fig 2.10c**.

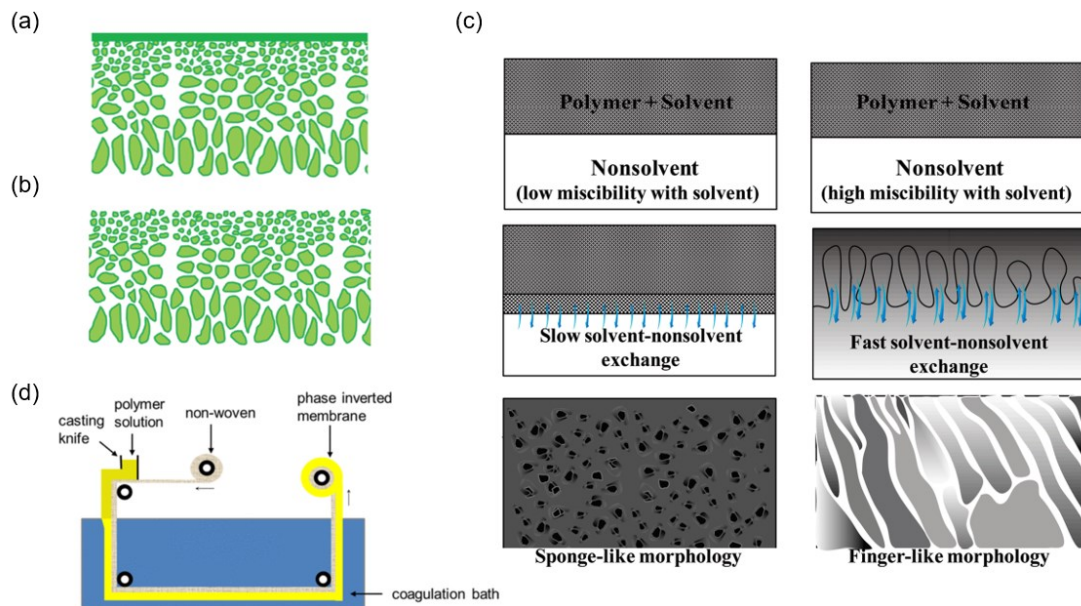


Fig. 2.10 (a) Schematic representation of a thin film composite membrane. (b) Schematic representation of an integrally skinned asymmetric membrane. Reproduced with permission from [9] (c) Different membrane morphologies caused by different types of demixing. Reproduced with permission from [10] (d) ISA membrane formation process by non-solvent induced phase inversion. Reproduced with permission from [11].

Phase inversion by immersion precipitation is well established, however, it is difficult to repeatedly manufacture a consistent membrane due to the number of critical variables in the casting process. These include [133,148,168–174]:

- Polymer: type, average molecular weight, polydispersity
- Dope formation: polymer concentration, solvent choice, co-solvents, non-solvent addition, additives
- Backing material: composition, pore size, roughness, hydrophilicity
- Casting conditions: non-solvent choice, non-solvent composition, temperature, humidity, cast thickness
- Post casting treatment: storage solvent, solvent exchange rate, time of storage

Each of these factors needs to be controlled to ensure the final membrane is consistent. A significant amount of literature has been published to hypothesise the effect of changing any of these parameters, however these correlations are often difficult to predict and often require experimental verification [133].

Scalability is a key advantage of immersion precipitation for the production of ISA membranes. The scale up of the immersion precipitation process can be achieved using a roll-to-roll continuous casting machine, with a casting knife or a slot dye [175], shown in **Fig 2.10d**. This method allows for large areas of membrane to be produced in a single batch and enables the membranes to be used at industrial scale. There are many additional variables which have to be optimised for the continuous casting process including: casting speed, solvent bath mixing rate, size of the non-solvent baths, winder tension. However, if these are kept consistent, a reliable membrane can often be produced.

2.6.3 Improving Membrane Stability

Once a membrane has been cast, it needs to be made solvent stable. All ISA membranes are soluble in the solvent used to make their dope solution, along with solvents with similar properties. For most ISA membranes, this means that the membranes will not be stable in strong polar solvents such as DMF, DMAc, and NMP. However, these are also the solvents used for many applications of the OSN

membranes, particularly in the petrochemical and pharmaceutical industries [176]. As such, polymeric membranes are often crosslinked for stability. Inevitably there are always exceptions. PEEK membranes are highly stable in strong polar solvents without crosslinking. However, this necessitates that PEEK membranes are cast from a highly acidic solution [158].

Crosslinking forms links between adjacent polymer chains. Not only does this provide chemical stability, but it can also suppress plasticisation, change the hydrophilicity, and tighten the selective layer of the membrane [151]. Chemical crosslinking is the most prevalent in OSN and has been used for many polymer types including PI, PBI, and PAN. This process often uses small reactive molecules to form covalent, ionic, or hydrogen bonds between adjacent polymer chains and prevents chain mobility in the membrane. Crosslinking conditions are important, and the crosslinking agent can be present in the casting solution, coagulation bath, vapor phase, or in a solvent media [151]. Crosslinking can also be achieved thermally, which has been used for PI and for polyaniline (PANi) membranes [175,177], or using UV, which has been used for PSf, PS and PI membranes [178,179]. Examples of these different crosslinking types are shown in **Table 2.4**.

Crosslinked PBI

For DSP synthesis, a tight OSU membrane must be manufactured which is stable in a wide range of polar solvents, acids, and bases. For tight OSU membranes, limited polymers have been studied, however PI [137], PAN [180], and PBI [135] have been shown to be promising materials.

Of these polymer types, PBI is a high-performance thermoplastic that is particularly interesting for OSU. PBI is the generic name of a class of polymers, but for this thesis PBI will specifically refer to Poly[2,2'-(m-phenylene)-5,5'-bisbenzimidazole], which is the only PBI that is currently commercially available in solution phase. This is sold under the trade name Celazole and produced by PBI Performance Products (USA). PBI is interesting for OSU as it is highly mechanically and thermally stable, and has been predicted to manufacture membranes with consistent long-term performance [181]. In its native form PBI has moderate solvent resistance and is stable in solvents such as IPA, ethanol,

and acetone, along with moderate strength acids and alkalis [182]. However, PBI can be crosslinked to improve the chemical stability

Table 2.4 A range of successful crosslinking conditions for gas separation, aqueous, and OSN membranes

Membrane Type	Crosslinking Conditions	Information / Performance	Ref.
PI	C2, C3, C6, and C8 diamines in methanol	1 - 8 LMHBar in DMF with MWCO between 250 and 400 g mol ⁻¹	[183]
PI	Hexanediamine in water	1.1 LMHBar in DMF with 95% methyl orange rejection (327 g mol ⁻¹)	[184]
PI	UV Crosslinking	0.3 LMHBar in DMF with 97% Rose Bengal rejection (974 g mol ⁻¹)	[178]
PI	UV Crosslinking	Improved H ₂ /CH ₄ separation factor	[185]
PI	Thermal	Improved resistance to plasticisation	[177]
PI	Thermal	Improved solvent stability, constant permeability	[186]
PBI	Glutaraldehyde (GA) in water	Stable in DMSO, NMP, DMAc 3.69 LMHBar in ethanol with 99% remazol brilliant blue rejection (627 g mol ⁻¹) 5.21 LMHBar in ethyl acetate with 90% eosin rejection (648 g mol ⁻¹)	[187]
PBI	p-xylylene dichloride	Swelling in DMAc and NMP 0.21 LMHBar in ethanol with 97.2% rose bengal rejection (974 g mol ⁻¹) 0.031 LMHBar in isopropanol with 99.1% rose bengal rejection (974 g mol ⁻¹)	[188]
PBI	Dibromoxylene (DBX)	7 LMHBar in DMF with 37% PEG 400 rejection, and close to 100% PEG 2000 rejection	[12]
PBI	Dibromobutane (DBB)	1 LMHBar in DMF with 10% PEG 400 rejection, and close to 100% PEG 8000 rejection	[12]
PBI	Trimesoyl chloride (TMC)	5.8 LMHBar in IPA with 99.6% to remazol brilliant blue rejection (627 g mol ⁻¹) 80.8 LMHBar in hexane with 92% L- α -lecithin rejection (758 g mol ⁻¹)	[189]
PBI	H ₂ SO ₄	Stable in DMAc, DMF, not NMP 3.5 LMHBar in methanol with 98% tetracycline rejection (444 g mol ⁻¹) 7.1 LMHBar in hexane with 99% of L- α -lecithin rejection (758 g mol ⁻¹)	[154]
PBI	K ₂ S ₂ O ₈	89.6 LMHBar in DMF with 84% rose bengal rejection (974 g mol ⁻¹) 0.15 LMHBar in DMF with 99% rose bengal rejection (974 g mol ⁻¹)	[182]
PBI	Polydopamine (PDA)	4 - 12 LMHBar in DMF with MWCO between 300 and 550 g mol ⁻¹	[190]
PAN	Hydrazine hydrate in water	2.3 LMHBar in ethanol with 99.9% Remazol Brilliant Blue R rejection (627 g mol ⁻¹)	[180]
PAN	Hydrazine monohydrate in ethanol	1.7 LMHBar in DMF with MWCO < 600 g mol ⁻¹	[191]
PANi	Dichloro-p-xylene	0.97 LMHBar in acetone with 99% styrene 400 g mol ⁻¹ rejection	[192]

PANi	Glutaraldehyde (GA) in water and HCl	1.1 LMHBar in acetone with 99% styrene 400 g mol ⁻¹ rejection	[192]
PIM	Trimesoyl chloride (TMC)	3.4 LMHBar in ethanol with 90% remazol brilliant blue R rejection (627 g mol ⁻¹) 12.4 LMHBar in acetone with 97% rose bengal rejection (974 g mol ⁻¹)	[193]

PBI has been previously used for aqueous nanofiltration and reverse osmosis [194], and was first crosslinked with p-xylylene dichloride to tailor the rejection and permeance [195,196]. Following this, it was proposed as an OSN membrane after crosslinking with dibromobutane (DBB), and dibromoxylene (DBX). The DBX crosslinked membrane showed superior performance and has since been used in further studies [12,197]. Since then, several other crosslinking methods have been used including p-xylylene dichloride, glutaraldehyde (GA), sulfuric acid, K₂S₂O₈, and trimesoyl chloride (TMC), with varying degrees of final membrane stability [154,182,187,189,198].

GA, p-xylylene dichloride, and sulfuric acid crosslinking are all promising techniques, however they did not produce a membrane that was fully stable across a wide range of strong polar organic solvents. Therefore DBB and DBX crosslinking are proposed to be better candidates for DSP synthesis. The disadvantage of these three crosslinking techniques is they require a long crosslinking reaction time [199], but after crosslinking, the PBI membrane is stable in strong polar solvents, strong acids, and strong bases, particularly those required for DSP synthesis [12]. Additionally, crosslinked PBI membranes have been shown to have long term stability in process operation [126,189]. However, published PBI membranes predominantly target a MWCO for OSN. This is too tight for DSP synthesis, and therefore, further tailoring of the rejection curve is essential.

2.6.4 Tailoring Performance

DSPs have strict selectivity requirements which necessitate > 99.5% rejection of the growing DSP at every iteration, and a low rejection of the unreacted monomers. The rejection properties can be controlled by the casting process. However, casting is difficult to control especially to the strict selectivity requirements that are needed for DSP synthesis. Crosslinking can also be used to control the

selectivity. However, crosslinking should usually be maximised, in order to maximise the stability of the final membrane and the reproducibility of the crosslinking process.

Another method of tailoring the membrane performance is using post casting modifications. Post casting modifications allow for the properties of the membrane to be tuned and tailored without affecting the initial cast. This allows for some of the membrane properties to be deconvoluted from the casting process [200–203]. A number of examples of membrane modifications are shown in **Table 2.5**.

One of the most common methods of modifying a membrane is the grafting of polymer chains to the surface of the membrane. UV irradiation, chemical reaction, or plasma treatment have been used for the grafting process. In aqueous systems, polymer graft modification has been used to tailor the fouling properties and rejection performance of the membrane. This is achieved by changing the effective pore size, hydrophilicity, charge, or surface roughness.

Grafting can be used to change the hydrophilicity and hydrophobicity of the membrane surface and promotes antifouling properties. Polymers for grafting include phospholipids, HEMA, PVDF, polyglycerol, and most commonly PEG [204–206]. Zanini et al. used PEG grafted onto PP membranes to reduce protein adsorption on the membrane by 95% [207]. Kurniawan et al. also used PEG grafted to cellulose membranes to decrease protein fouling by more than 70% [208]. Chinpa et al. modified PEI membranes with PEG. They achieved a change in the contact angle from 83.1° before treatment to 57.8° after treatment, but with no corresponding decrease in the pore size [209]. The use of PEG is common in aqueous systems because it forms hydrogen bonds with water, resulting in a high hydrodynamic radius [210]. In organic systems, Van Gestel et al. showed that grafting with C8 silane or C1 silane changed the hydrophilicity of the membrane and changed the MWCO in hexane from 410 to 650 [211]. Rezaei Hosseinabadi et al. used alkyl chains to surface functionalise ceramic membranes. This increased the hydrophobicity of the membrane and increased the rejection [143].

Grafting can also be used to alter the charge of a membrane. To modify the membrane charge, zwitterionic polymers have been used including phosphatidylcholine, phosphobetaine, sulfobetaine,

and carboxybetaine [203]. Other methods of adjusting the charge are treatment with acids and amines [212,213].

Table 2.5 A range of successful modification techniques for aqueous and OSN membranes.

Membrane Type	Surface Modifier	Grafting Initiator	Information / Performance	Ref.
Crosslinked PI	PEG	Plasma	Conversion from UF membrane to NF 5.91 LMHBar in IPA with 99.64% rose bengal rejection (974 g mol^{-1})	[214]
Polyamide and Polyester	PEG	Plasma	Reduced biofilm formation by 96%	[215]
Polysulfone	PEG	Plasma	Increased membrane hydrophilicity	[216]
Polysulfone	Hydroxyl, carbonyl, carboxylic acid groups	Plasma	Increased hydrophilicity. BSA adsorption reduced to less than half of the control membrane	[217]
Cellulose	PEG	Plasma and Chemical	Improved resistance to cell adhesion using plasma (30%) Improved resistance to cell adhesion using chemical grafting (70%)	[208]
PVDF	Zwitterinoic poly(sulfobetaine methacrylate) (PSBMA)	Chemical	Increased fouling resistance	[218]
Ceramic TiO ₂	Perfluoroalkyl silanes	Chemical	Increased mechanical resistance and hardness	[219]
Ceramic	Alkyl groups (C1, C5, C8, C12)	Chemical	Increased hydrophobicity, increased rejection	[143]
Poly-propylene	Polyvinyl alcohol/4-vinylpyridine	Chemical Adsorption	Improved antifouling properties, decrease in permeate flux	[220]
PES	Polyethylene glycol methacrylate	UV irradiation	Increased resistance to fouling, increased rejection	[221]
PVDF	Acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA)	UV irradiation	Increased resistance to fouling, increased membrane hydrophilicity	[222]
PAN	HEMA	UV irradiation	Increased resistance to fouling and adsorption	[223]

Most importantly, the rejection of the membranes has been shown to be dependent on the grafting. Gao et al. grafted polyethylene glycol chains to a PI membrane to increase the rejection of Rose Bengal dye from 70.1% to 99.6% [214]. Gassara et al. grafted mono and poly aminated oligomers onto polyether imide (PEI) membranes to control the pore size between 0.3 nm and 11 nm, depending on reaction time and grafting agent [224]. These studies show the flexibility and effectiveness of polymer grafting as a technique for tuning the rejection profile, anti-fouling properties, and charge of membranes.

2.6.5 PBI for DSP synthesis

In order to manufacture a membrane for DSP synthesis, the membrane must be mechanically, thermally, and solvent stable over long term operation. PBI is highly thermally and mechanically stable. Additionally, it has been shown to have high stability in a wide range of organic solvent, acids, and bases after crosslinking with DBX, and is therefore a highly promising candidate for manufacturing a membrane for DSP synthesis [12].

For PBI membranes, previous studies have found that tuning the rejection profile of PBI is difficult [225]. Using a post-crosslinking modification step could enable the rejection profile of the PBI to be tailored for DSP synthesis, and graft modification has been shown to be a highly flexible and effective method of modifying membranes [224]. This is an important area of development towards the optimisation of a membrane for DSP synthesis, and therefore towards the success of membrane enabled liquid phase synthesis as a platform technology for DSP manufacture.

Chapter 3: Research Objectives

The literature review shows the significant limitations of solid phase synthesis (SPS) for manufacturing defined sequence polymers (DSPs). Conversely, liquid phase synthesis (LPS) is a potentially high purity and scalable method of synthesising high purity DSPs. However, with LPS, it is difficult to achieve high yield and high purity separations. Coupling LPS to organic solvent stable membrane separations is a promising opportunity for DSP manufacture. Here, four main research objectives are described which would enable the successful synthesis of DSPs using membrane enabled LPS.

Objective 1: Develop a solvent stable membrane for DSP synthesis

The main barrier to the successful manufacture of DSPs using membrane separations is achieving a good separation. However, there are few commercial membranes which are suitable for DSP synthesis, as most commercial solvent stable membranes target a molecular weight cut off (MWCO) below 1000 g mol⁻¹. DSP synthesis requires a significantly higher MWCO, between loose organic solvent nanofiltration (OSN) and tight organic solvent ultrafiltration (OSU), and therefore requires the development of a new membrane. The first objective of this work was to manufacture a solvent stable membrane which targets 100% rejection of the growing DSP and 0% rejection of the reaction debris and unreacted building block.

This membrane development investigated polybenzimidazole (PBI), due to the proven solvent stability of PBI membranes after crosslinking with dibromoxylene (DBX). Due the broad range of potential DSP types, the first target of this objective was to develop a method of customising the rejection profile of crosslinked PBI. This customisation focused on using graft modification, which was shown in the literature review to be an effective method of tailoring the rejection profile of a membrane. The second target of this objective was to scale up the graft modified PBI synthesis. This required the scale up of the casting, crosslinking, and graft modification steps, ensuring that the performance after scaling up was consistent with the performance at small scale.

The following research strategy was used in **Chapter 4** to achieve this objective:

- Develop a method of graft modifying PBI
- Investigate graft modification as a method of controlling the membrane rejection profile
- Crosslink and graft modify sections of PBI at meter length scale with achieve consistent performance to small scale PBI production
- Manufacture spiral wound modules with consistent performance to flat sheet cells

Objective 2: Improve the long-term performance of PBI membranes

Polymeric membranes for nanofiltration and ultrafiltration are commonly prepared through non solvent induced phase separation (NIPS). This process solidifies the polymer chains in a non-equilibrium state and creates an excess free volume which forms the permeation pathways in the membrane. However, this excess free volume makes polymeric membranes susceptible to polymer chain rearrangement and ageing. This decreases the efficiency of the membrane separation and has a significant impact on the economics of the process.

The second objective was to investigate the long-term process stability of PBI membranes. This objective focused on minimising the mobility of the polymer chains by investigating the effect of the extent of crosslinking and the polymer molecular weight. This was essential to ensure that the membranes were fully process stable for DSP manufacture.

The following research strategy was used in **Chapter 5** to achieve this objective:

- Manufacture PBI membranes from different polymer molecular weights and different crosslinking conditions
- Investigate the long-term process stability of PBI in different solvents
- Maximise the long-term stability of the PBI membrane

Objective 3: Develop an antifouling solvent stable membrane

Fouling is inevitable in all membrane-based separation processes. Fouling is the accumulation of particles or molecules on the top surface, or inside the pores of a membrane. These particles lead to the decline of the permeance, and often also the selectivity, of a membrane over time. In aqueous systems,

fouling is well characterised and fouling reduction techniques are established. However, in organic solvents, membrane fouling is not well understood, and anti-fouling membranes do not yet exist. The development of anti-fouling membranes for organic solvent separations will lead to a reduction of energy, maintenance, cleaning, and labour cost for operating OSN and OSU processes.

In aqueous systems, graft modification with polymer chains provides an effective method of reducing fouling. This is partly due to a steric repulsive effect which prevents adsorption of particles to the surface of the membrane. The third objective investigated the use of graft modification to manufacture anti-fouling membranes for organic solvent separations. This objective focused on using PBI with a range of different graft modifiers and measuring the performance and anti-fouling properties of these membranes. The target was to investigate correlations between the properties of the graft modifier and the properties of the solvent to optimise the anti-fouling properties of grafted PBI membranes in different solvents

The following research strategy was used in **Chapter 6** to achieve this objective:

- Fabricate PBI membranes with different length and hydrophilicity graft modifiers
- Investigate the effect of different graft modifiers on membranes performance
- Investigate the effect of different graft modifiers on membranes fouling
- Examine the anti-fouling properties of graft modified membranes in different solvents

Objective 4: Synthesise an industrially relevant DSP

In order to prove the capabilities of membrane enabled liquid phase synthesis as a new platform for DSP manufacture, a commercially relevant DSP must be synthesised. While oligonucleotides are difficult DSPs to manufacture at large scale, the current therapeutic pipeline of oligonucleotide DSPs requires a significant increase in production capacity. Therefore, a pharmaceutical length oligonucleotide is an ideal molecule to test the capabilities of membrane enabled liquid phase DSP synthesis.

The fourth objective was the synthesis of a pharmaceutically relevant length oligonucleotide using membrane enabled liquid phase synthesis. The main benefits of this methodology are scalability, automatability, and an entirely liquid phase process. Therefore, one of the aims of this objective was to design an oligonucleotide synthesiser which utilises those benefits. This required construction of a well-mixed single apparatus synthesiser, which can perform the entire synthesis without removing the material from the apparatus. This enables fast, automated, and scalable synthesis. The second aim was to design and optimise a soluble hub molecule, which must be optimised in parallel with the organic solvent stable membrane, to ensure 100% rejection of the oligonucleotide and achieve a high final product yield. The final aim was the synthesis of a pharmaceutical length oligonucleotide at comparable purity and yield to the current industrial standard, SPS.

The following research strategy was used in **Chapter 7** to achieve this objective:

- Design and build a single apparatus, single phase oligonucleotide synthesiser
- Optimise a soluble hub molecule to maximise the oligonucleotide yield in the synthesis
- Synthesise a pharmaceutical length oligonucleotide

Chapter 4: Graft modification of polybenzimidazole membranes for organic solvent ultrafiltration with scale up to spiral wound modules

Abstract

In this chapter, the selectivity of crosslinked organic solvent stable PBI membranes was tuned by varying the molar ratio of two grafting agents: a long flexible polymer modifier (1000 g mol^{-1} PEG) and a small molecule modifier (2-methoxyethylamine, 75 g mol^{-1}). Grafting exclusively with the long chain modifier provided a membrane with a molecular weight cut-off (MWCO) of $2,000 \text{ g mol}^{-1}$ in acetonitrile. Increasing the proportion of small molecule modifier in the grafting mixture capped the grafting sites in the membrane. This caused a decrease in the grafting density of the polymer modifier and allowed for the MWCO to be customised between $2,000 \text{ g mol}^{-1}$ and $20,000 \text{ g mol}^{-1}$ in acetonitrile. High grafting densities underlie this control of performance, with up to 43% increase in the specific weight of the membrane during modification. This technique was scaled up to the manufacture of 5 m lengths of membrane that were incorporated into 1.8" x 12" spiral wound modules, which showed performance consistent with the flat sheet membranes.

Keywords: Organic solvent nanofiltration, Polybenzimidazole (PBI), Graft modification, Spiral wound module, Organic solvent ultrafiltration

Highlights

- Crosslinked polybenzimidazole (PBI) membranes have high stability in harsh solvents
- Graft modification allows for customisation of the rejection profile of PBI
- Graft modifier is present throughout the permeation pathways of the membrane
- The resulting membranes span from loose nanofiltration to tight ultrafiltration
- The membranes have consistent performance as flat sheets and spiral wound modules

4.1 Introduction

The use of organic solvent stable membranes for DSP synthesis requires tuning the selectivity of a membrane. The membrane must have a high rejection of the growing DSP, while permeating the smaller reagents. The membrane must also have high flux, and it must be possible to consistently produce defect-free membrane at the required scale. Above all, the membrane must have long-term stability in the process solvent [11].

Research into organic solvent stable membranes starts with material selection. Polybenzimidazole (PBI) has recently attracted significant attention for OSN due to its outstanding mechanical and thermal stability [154]. It can also be made chemically stable with post-casting modifications such as interpenetrating polydopamine [190], or using crosslinkers such as *p*-xylylene dichloride [188] or trimesoyl chloride [189]. Valtcheva et al. [12] first explored α,α' -dibromo-*p*-xylene (DBX) as a crosslinking agent for PBI. Crosslinking with DBX created a membrane which was highly stable over a wide range of pH (0 to 14), and in polar and nonpolar solvents. This membrane was shown to be effective in numerous applications including cascade operations [126], peptide synthesis [99] and oligonucleotide synthesis [7]. However, it was found that the selectivity of the PBI membranes was difficult to tailor [225].

Successful purifications with membranes require tuning the membrane selectivity. Common methods for tuning the selectivity of integrally skinned asymmetric membranes are either to change the membrane pore structure or surface interactions [150]. This can be achieved through changing the dope composition, using dope additives, or altering casting conditions [133]. However, researchers still struggle to predict reliably the outcome of the phase inversion processes for many polymers, and the tools to mitigate the formation of non-uniform pore size distributions do not yet exist, macrovoids and defects [226].

An alternative method for tuning the selectivity of a membrane is to use post-casting modifications [227]. These modifications preserve the underlying membrane morphology, in principle potentially

decoupling the casting of the membrane from the membrane selectivity. One widely researched post-casting modification is the covalent grafting of polymer chains to the membrane surface.

Polymer grafting can change the membrane selectivity, hydrophilicity, and surface charge [228]. There are two different techniques for attaching polymers to the membrane: grafting-from or grafting-to. Grafting-from involves initiation, followed by subsequent polymerisation on the surface of a membrane. UV radiation, ozone, and high energy radiation [229–231] have all been used for the initiation. However, the harsh grafting-from conditions have been observed to change the morphology of the top selective layer [201]. This technique provides high grafting densities, but the length of the grafted polymers is inconsistent and difficult to characterise.

Grafting-to reacts polymers of a predefined length with the membrane surface. Shen et al. used grafting-to of different molecular weight polyethylene glycols (PEG) to modify PVDF/SMA blend membranes. They found that the molecular weight impacted grafting density, and the combination of these two factors changed the pore size, rejection and permeance [232]. Gassara et al. grafted monoaminated and polyaminated oligomers onto polyether imide (PEI) membranes to control the pore size between 0.3 nm and 11 nm, depending on reaction time and grafting agent [224]. Rezaei et al. used Grignard chemistry to graft alkyl groups (methyl, pentyl, octyl, dodecyl) to TiO₂ ceramic membranes to enhance the surface hydrophobicity. The variable rejection behaviour was hypothesised to be a function of the tuneable solute-solvent-membrane interactions [139]. Because the polymers are of a known length, grafting-to techniques provide a much more consistent method for tuning the membrane. However, it is difficult to achieve high grafting densities, and grafting processes often require additional reaction steps to introduce reactive sites into the membrane [201].

PBI membranes crosslinked with DBX are ideally suited for tuning the membrane selectivity by grafting-to modification. The crosslinking of PBI membranes has been shown to result in up to 20% bromine content inside the membrane, depending on the crosslinking conditions [199]. This bromine content is associated with unreacted functional groups on the DBX crosslinker molecule. Such reactive sites are believed to be present throughout the entire cross section of the membrane, including in the

permeation pathways. Using a grafting-to technique would react long polymeric chains to the polymers bounding the permeation pathways and thus, increase the flow resistance inside the pathways. This modification step would change the solute-solvent-membrane interactions but would be fully independent from the crosslinking reaction, ensuring that the solvent stability remains unchanged.

In this study the residual reactivity of DBX-crosslinked PBI is exploited by modifying these membranes with a long linear monoaminated polymer - Elastamine RE1-1000 (1kPEG), consisting of a mixture of polyethylene glycol (PEG) and polypropylene glycol (PPG) repeating units at an average molecular weight of 1000 g mol⁻¹. The degree of modification is controlled using a competing reaction with 2-methoxyethylamine, a small 75 g mol⁻¹ monoamine, whose presence reduces the extent of modification by 1kPEG. This control allows customisation of the rejection profile to generate a series of PBI membranes that have a molecular weight cut off (MWCO) ranging from tight ultrafiltration to loose nanofiltration. These membranes are excellent candidates for purification processes in which it is desirable to permeate one or more smaller compounds (500 - 2,000 Da) while retaining a larger molecule (2,000 – 20,000 Da). These robust membranes have been validated in a recent publication on liquid phase peptide synthesis [99]. Furthermore, the scalability of this process has been demonstrated by the manufacture of defect-free 1.8” x 12” spiral wound modules.

4.2 Materials and Methods

4.2.1 Materials

Celazole S26 Polybenzimidazole (26 wt% PBI and 1.5 wt% lithium chloride in N,N-dimethylacetamide (DMAc)) was purchased from PBI Performance Products, Inc (USA). Non-woven support Novatexx 2471, made of polypropylene, was purchased from Freudenberg Filtration Technologies (UK). HPLC grade acetonitrile (MeCN) and propan-2-ol (IPA) for membrane synthesis were purchased from VWR (UK). N,N-dimethylacetamide, α,α' -dibromo-*p*-xylene (DBX), 2-methoxyethylamine (2-MEA), N,N-diisopropylethylamine, and PEG (400 g mol⁻¹) for membrane synthesis were purchased from Sigma Aldrich (UK). Elastamine RE1-1000 (1kPEG) was kindly donated by Huntsman (UK). PEG (400, 1000, 2000, 8000, and 35000 g mol⁻¹) for membrane testing was purchased from Sigma Aldrich (UK). Other

reagents (acetonitrile, chloroform, dimethylformamide (DMF), methanol, toluene, water, HCl, NaOH) were all purchased from VWR (UK).

4.2.2 Methods

4.2.2.1 Fabrication

Celazole S26 was diluted to 18 wt% polymer in DMAc and continuously stirred to form a homogenous dope. The solution was then filtered through nylon 11 μm filter paper (Millipore) and allowed to stand for 24 h to remove air bubbles. The dope was cast on polypropylene backing using a continuous casting machine (SeptraTek, Korea) with a knife height of 0.2 mm and a casting speed 1.4 cm s^{-1} . The phase inversion was conducted in DI water at $20\text{ }^{\circ}\text{C}$. The membranes were then stored in DI water for 48 h, exchanging to fresh DI water after 24 h. The membranes were then washed three times in acetonitrile, stirring to remove residual solvent and water.

4.2.2.2 Crosslinking

The crosslinking reaction was performed under an argon atmosphere. For small-scale crosslinking, a $0.2\text{ m} \times 0.3\text{ m}$ membrane roll was placed in acetonitrile in a 160 mL reaction cylinder. For large-scale crosslinking, $5.0\text{ m} \times 0.3\text{ m}$ of membrane roll was placed in acetonitrile in a 4 L reaction cylinder. 0.08 mol L^{-1} DBX was added and maintained at $70\text{ }^{\circ}\text{C}$ for 24 h. The solution was then cooled to room temperature and the membrane washed three times with fresh acetonitrile.

4.2.2.3 Modification

After crosslinking, the PBI was immersed in acetonitrile containing a hindered base, N,N-diisopropylethylamine (0.6 mol L^{-1}). 1kPEG and 2-MEA (at a total concentration of 0.4 mol L^{-1}) were added with the ratio designated in **Table 4.1** for each membrane synthesis. In all cases, the solution was heated at $70\text{ }^{\circ}\text{C}$ under an argon atmosphere. After 24 hours the solution was cooled to room temperature and the membrane washed three times with fresh acetonitrile.

4.2.2.4 Preservation

The membrane was immersed in a mixed solution of PEG (400 g mol⁻¹):IPA 1:1 (v/v) for 24 hours at room temperature with constant stirring. The membrane was then removed from the solution and dried at room temperature in a fume cupboard.

Table 4.1 Summary of the membrane modifications. The naming system and modification ratio are described.

Name	Condition	1kPEG (mol%)	2-MEA (mol%)
PBI	Uncrosslinked	-	-
PBI - DBX	Crosslinked	0	0
M - 0	Crosslinked and modified	0	100
M - 90	Crosslinked and modified	90	10
M - 97.5	Crosslinked and modified	97.5	2.5
M - 100	Crosslinked and modified	100	0

4.2.2.5 Spiral Module Production

For the spiral wound modules, ~1.7 m x 0.2 m sheets of PBI were interleaved with a polypropylene feed spacer (0.7 mm thickness) and polypropylene permeate spacer (0.298 mm thickness). This sandwich was wrapped around a central collection tube and sealed with an epoxy-based adhesive (supplied by Evonik MET). The module was cured at 75 °C for 19 h.

4.2.2.6 Characterisation

For elemental analysis, the membranes were washed three times with acetonitrile, then dried in a vacuum oven overnight at 80 °C and 300 mbar (Fistreem). The membranes were then peeled off the polypropylene backing. The carbon, hydrogen and nitrogen content were measured by combustion analysis (CE instruments, EA1100). The bromine content was measured by oxygen flask combustion followed by ion chromatography (Dionex ICS 1000).

The specific weight change of the membranes was calculated from weighed discs having an area of 20 cm² which had been washed with acetonitrile and dried in a vacuum oven overnight at 80 °C, 300 mbar (Fistreem). A minimum of four discs of each type of membrane were weighed.

For scanning electron microscopy (SEM), samples of PBI-DBX, M-100, and M-0 were freeze fractured in liquid nitrogen. Approximately 5 nm of gold nanoparticles were sputtered onto the cross section of each membrane. An FEI Inspect F50 was used for analysing the samples with an accelerating voltage of 10 kV.

Samples for atomic force microscopy (AFM) were dried and then measured on a Bruker Dimension Icon using a RTESPA-300 Cantilever (40 Nm⁻¹). Images were measured at 256 x 256 pixels with a scan rate of 1 Hz and a peak force frequency of 2 kHz. Images were processed in Nanoscope Analysis.

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) was carried out using an IONTOF ToFSIMS 5 instrument. For analysis of the cross section in the ToF-SIMS, samples of M-100 and PBI-DBX membrane were cross sectioned using a scalpel blade. The freshly cut sections were then placed in a metal clip in order to ensure that the cross sectioned membranes were kept straight and flat in the ion beam and time of flight analyser to ensure good secondary ion transmission. For top surface analysis, the samples were clamped flat onto the stage. Before ion mapping the samples were cleaned using an Ar cluster ion beam (10 keV Ar₁₀₀₀⁺) over a 1000 µm² area. This removed any extrinsic hydrocarbons that would be on the samples. A 25 keV Bi₃⁺ ion beam was then used in burst alignment mode (~0.1 pA ion beam current) to ion map the cross sections of the samples.

Thermogravimetric analysis (TGA) was conducted using a Perkin Elmer TGA 8000. Approximately 5 mg of dry sample was preheated at 100 °C in a nitrogen atmosphere for 20 minutes. The temperature was then scanned from 100 °C to 1000 °C at a rate of 5 °C min⁻¹ in a nitrogen atmosphere.

Contact angle was measured on a Kruss DSA. 5 µL water was deposited onto the surface of each membrane and a minimum of ten measurements were taken for each membrane.

4.2.2.7 Stability Test

20 cm² discs of membrane were taken post modification, washed with acetonitrile three times and dried in a vacuum oven overnight at 80 °C and 300 mbar (Fistreem). The initial weight was recorded, and then the samples were placed into solvent solutions for two weeks. These solutions were DMAc, toluene, acetonitrile, DMF, chloroform, 20 wt% HCl in water and 20 wt% NaOH in water. The samples were then washed three times with acetonitrile, dried in a vacuum oven overnight and weighed again. The weights were compared to the initial values.

4.2.2.8 Performance

Membrane performance was determined by filtration experiments. The performance tests were conducted on an eight-cell cross-flow rig, split into two parallel paths of four membrane cells each, as used by da Silva Bural et al. [158]. Membrane discs with an active area of 14 cm² were used and a minimum of four discs of each membrane were tested. The spiral wound module tests were undertaken on the same rig. The rig was operated at 75 L h⁻¹ cross-flow rate per parallel path, 10 bar and maintained at 30 °C. Prior to determining the permeance and rejection, the membranes were compacted at 10 bar for a minimum of 24 hours.

Pure solvent permeances were taken, followed by addition of 1 g L⁻¹ each of five poly(ethylene glycol) with various molecular weights. These were PEG 400, 1000, 2000, 8000, and 35000 g mol⁻¹ and the total concentration of solute in the system was 5 g L⁻¹. The samples were analysed using an Agilent HPLC coupled with an evaporative light scattering detector (ELSD Varian-385). A reversed phase Hichrom Ace column (C18-300, 250 mm 4.6 mm I.D.) was used. Two mobile phases were utilized. Mobile phase A was 5 mM ammonium acetate aqueous solution and mobile phase B was 4:1 (v:v) acetonitrile-methanol mixture. The gradient was 2 minutes isocratic 90% A, followed by a linear ramp to 5% A over 23 minutes. The system was then decreased back to 90% A over two minutes and re-equilibrated with 90% A for 5 minutes prior to the next injection. A flow rate of 1 mL min⁻¹ and a column temperature of 30 °C were used. The ELSD evaporator was set at 40 °C and the nebulizer at 55 °C. The coefficient of variation of this analysis at 1 g L⁻¹ of each solute was less than 1.2%.

The permeance, B ($\text{L m}^{-2} \text{h}^{-1} \text{bar}^{-1}$), and the rejection of component i , R_i , were calculated using Equations (1) and (2) respectively:

$$B = \frac{V}{A \cdot t \cdot \Delta P} \quad (1)$$

$$R_i = 1 - \frac{C_{p,i}}{C_{F,i}} \quad (2)$$

where V (L) is the volume collected, A (m^2) is the effective membrane area, t (h) is the time over which the sample is collected, Δp (bar) is the applied trans-membrane pressure and $C_{p,i}$ and $C_{F,i}$ (mol L^{-1}) represent the concentration of solute i in the permeate and the feed, respectively. The osmotic pressure was assumed to be negligible due to the low concentration of solute.

4.2.2.9 Graft Location Modification

To determine the location of grafting, crosslinked PBI membranes were modified inside crossflow cells. PBI membranes were placed on top of an impermeable polypropylene (PP) sheet to ensure the modifying solution could only access the PBI membrane from one direction. The PBI was either placed with the selective layer facing the modifying solution (top modification) or with the selective layer facing the PP sheet (bottom modification). Throughout the modification, there was no permeation through the impermeable PP sheet. The M-100 modifying solution was placed in a feed flask and circulated through two 51 cm^2 crossflow cells in series using a HPLC pump (Gilson 305). The rig was operated at a flow rate of 100 ml min^{-1} . The temperature of the rig was maintained at 70°C using heat exchangers before each cell connected to a heater (Lauda L100). The feed flask was also heated to 70°C in an oil bath.

For each case, a minimum of four cells were modified. From each cell, a 14 cm^2 disc was cut and tested, as described in **Section 4.2.2.8**, and the top surface of the rest of the disc was analysed by TOF-SIMS, as described in **Section 4.2.2.6**. The top surface of PBI-DBX and M-100 membranes, which had been modified with the standard methodology, were also analysed by TOF-SIMS.

4.3 Results and Discussion

4.3.1 Crosslinking and Modification

Fig. 4.1 is a schematic illustrating the fabrication of the membranes described in this chapter. The crosslinking process involves a two-stage reaction. The first stage was electrophilic alkylation of the PBI polymer chains with DBX. The rate of the initial reaction of the first bromomethyl group of DBX with a PBI chain (**Fig. 4.2**, Reaction 1) competes with the rate of the chain cross-linking reaction by the second bromomethyl moiety (**Fig. 4.2**, Reaction 2). If all the sites on neighbouring PBI chains within range of a singly reacted DBX have been consumed before it can undergo the second reaction, then this will leave residual reactive bromomethyl groups, or grafting sites, within the membrane matrix. Due to the high concentration of dissolved DBX in the bulk solution, this competition will favour grafting sites at the very top surface, declining as the concentration of free DBX diffusing deeper into the surface declines. These reactive grafting sites provide locations for modification of the membrane surface.

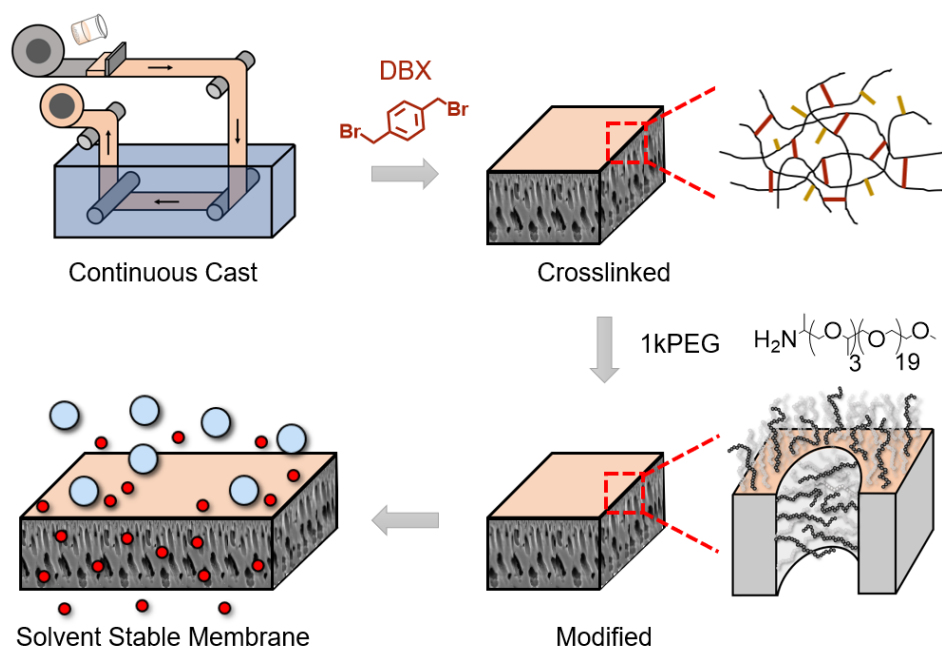


Fig. 4.1 Three-step manufacturing process to produce solvent stable PBI membranes.

To control the rejection profile, the modification process requires the parallel addition of 1kPEG and 2-MEA. These monoamines react with any grafting sites present in the membrane. This is a competitive reaction between the long chain modifier (1kPEG, 1000 g mol⁻¹) and the short chain modifier (2-MEA,

75 g mol⁻¹), as shown in **Fig. 4.2** (Reaction 3). The proportion of modification by each species is significantly dependant on the molar ratio of the two species in solution, although other factors will also affect their relative rates of reaction, such as molecular weight dependent speeds of diffusion and steric compression. The different molar ratios for modification conditions are shown in **Table 4.1**.

An additional advantage to this method is that the modification process will simultaneously passivate the membrane. The presence of free bromomethyl sites provides a highly reactive species which could react with permeating solutes or solvents. This might cause unpredictable changes to the membrane performance over time and lead to inconsistencies in the separation process. Removal of the reactive bromomethyl groups passivates the membrane and should lead to more consistent performance.

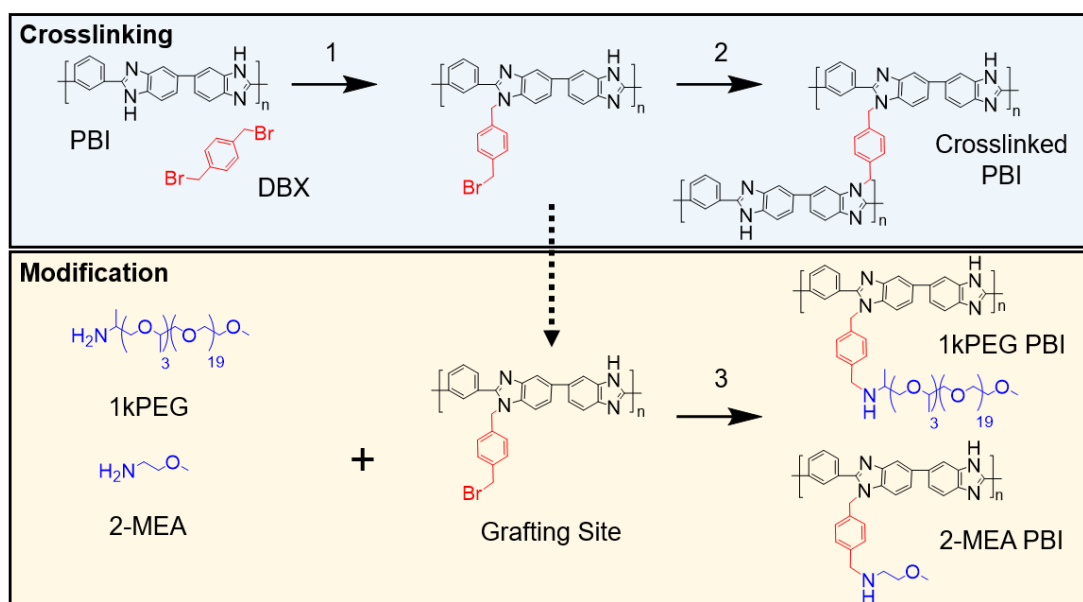


Fig. 4.2 Proposed crosslinking and modification reactions on one of the reactive sites of PBI-DBX. Modifications are shown with 1kPEG or 2-MEA.

4.3.2 Characterisation

The physico-chemical properties of the membranes were quantified by elemental analysis, weight change, TGA, SIMS, SEM, and AFM.

As shown by the elemental microanalysis in **Table 4.2**, the crosslinked membrane (PBI-DBX) had a high Br/N ratio, of 0.50. Upon modification with 1kPEG, the bromine content of the M-100 membrane

decreased significantly to a Br/N ratio of 0.29. This was consistent with successful modification of the membrane, but the M-100 membrane still contained some residual bromine. This could be due to the inability of large 1kPEG polymer molecules to pack close enough together to consume all the available grafting sites, or the difficulty for 1kPEG polymer molecules to access very tight pores. Upon the addition of 2-MEA to the modification reaction (M-97.5, M-90 and M-0), the Br/N ratio decreased slightly. This is believed to be due to the smaller size of 2-MEA which allows it to diffuse into the tighter pores, and between large 1kPEG molecules, and therefore to react with a higher percentage of the residual bromine than the 1kPEG. The 2-MEA is in molar excess to the number of brominated sites in all modification reactions, so the end point of the modification always tends to the same Br/N ratio.

After modification with 1kPEG (M-100), the C/N ratio and the H/N ratio increased relative to the unmodified membrane (PBI-DBX), increasing from 5.6 to 6.8 and from 0.39 to 0.63 respectively. This is due to a higher C/N ratio and H/N ratio in the 1kPEG polymer than the crosslinked PBI, and this demonstrates successful grafting with 1kPEG. For the grafted membranes, the C/N ratio and H/N ratio follows the order M-100 > M-97.5 > M-95 > M-0. The 1kPEG polymer has a higher C/N ratio and H/N ratio than 2-MEA. This means that the 1kPEG grafting will result in the largest change in the C/N and H/N ratio, with the 2-MEA having little effect on the C/N and H/N ratio. Therefore, the quantity of 1kPEG grafted to the membranes will follow the order M-100 > M-97.5 > M-95 > M-0.

Table 4.2 Elemental analysis of unmodified and modified membranes relative to the nitrogen content of each membrane.

	C/N Ratio	H/N Ratio	Br/N Ratio
PBI-DBX	5.6	0.39	0.50
M-0	5.3	0.41	0.24
M-90	6.0	0.53	0.24
M-97.5	6.4	0.57	0.24
M-100	6.8	0.63	0.29

The specific weight change of the membrane was used to quantify the ratio of 1kPEG to 2-MEA reacting with the membrane, as shown in **Fig. 4.3a**. Modification with the 1kPEG polymer will cause an increase in the specific weight of the membrane. In contrast, modification with a small molecule, such as 2-MEA, will lead to negligible mass change. The highest weight change was 14.4 g m^{-2} and occurred in the M-100 membranes, which corresponded to a 43% weight increase of the PBI-DBX membrane. The weight change decreased as the molar percentage of 1kPEG in the modification reaction was reduced. This is assumed to be due to the 2-MEA out-competing slow and hindered 1kPEG for accessible bromomethyl sites. M-97.5 showed a 75% weight change relative to M-100, suggesting 75% saturation of the accessible sites by 1kPEG. M-90 showed 50% relative weight change compared to M-100, suggesting 50% saturation with 1kPEG. These findings demonstrate that varying the ratio of the two modifiers gives good control over the extent of long chain modifier coverage.

To demonstrate that the weight change is due to grafting of 1kPEG, the thermal decomposition temperature of the membranes was measured using TGA, **Fig. 4.3b**. The 1kPEG polymer had a decomposition temperature between 250°C and 450°C . In this range, the 1kPEG modified membrane (M-100) showed the largest weight change, corresponding to a membrane with 41% greater mass than PBI-DBX. This correlates closely with the increase in the specific weight of the membranes due to grafting. For the other membranes, the weight change in the region of 1kPEG decomposition also matches the correlation from the specific weight change, following the trend, $\text{M-100} > \text{M-97.5} > \text{M-90} > \text{M-0}$. The M-0 modified membrane had practically no weight change, and this was due to the low molecular weight of the small molecule modifier, 2-MEA.

The contact angle of the membranes was used to demonstrate that the 1kPEG was grafted to the top surface of the membranes. 1kPEG is a PEG based polymer and is relatively hydrophilic. With 50% saturation of the grafting sites in the membrane with 1kPEG, the water contact angle decreased from 58° (M-0) to 47° (M-90), shown in **Fig. 4.3c**. Increasing the extent of grafted 1kPEG caused the water contact angle to consistently decrease. This resulted in a minimum water contact angle of 43° ,

corresponding to 100% 1kPEG saturation (M-100). This gives further evidence that the 1kPEG is bound to the surface of the membrane.

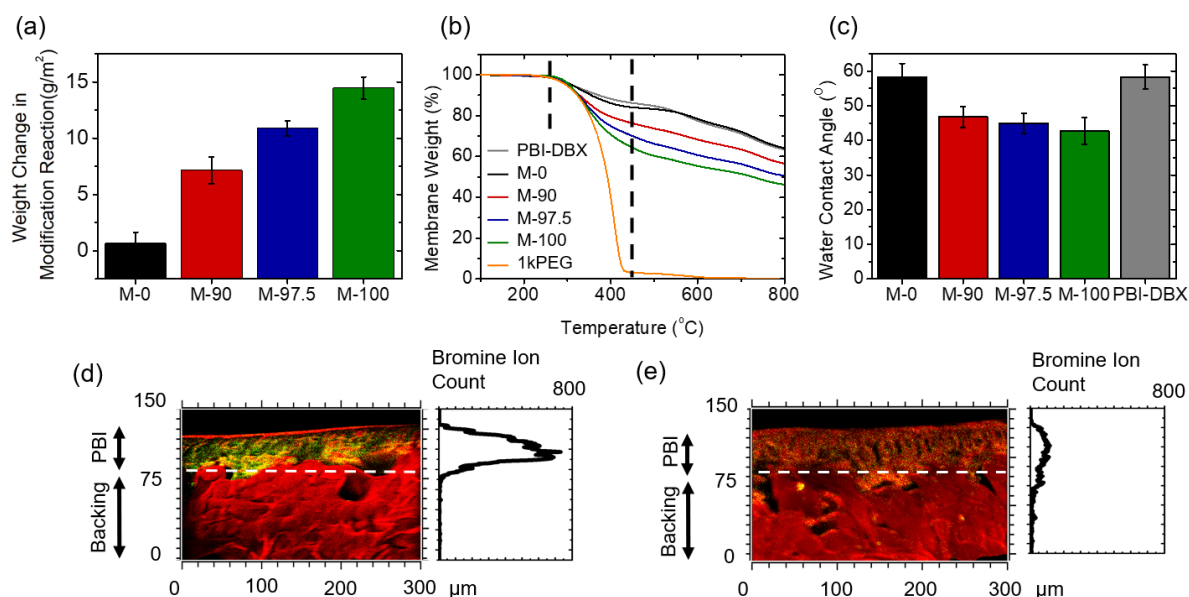


Fig. 4.3 (a) Specific weight change of the membranes after the modification reaction, relative to PBI-DBX. (b) TGA of the membranes before and after modification, as well as the pure polymer 1kPEG. The vertical dashed lines show the degradation range of 1kPEG. (c) Water contact angle of the membranes. (d) TOF-SIMS image of PBI-DBX. Bromine ions are highlighted in green, relative to all other ions shown in red. The dashed line differentiates the PBI membrane from the PP backing material. The vertical line scan of the bromine ion count is shown on the right. (e) TOF-SIMS image of M-100.

In order to investigate if the location of the grafting was just on the top surface of the membrane, the unmodified membrane, PBI-DBX, and modified membrane, M-100, were analysed for bromine content by TOF-SIMS, as shown in **Fig. 4.3d** and **e**. Without modification, there was a strong bromine intensity throughout the PBI-DBX cross section, with negligible amounts in the polypropylene backing. By contrast, after modification there was a reduction in bromine intensity across the whole membrane structure, including in the uppermost selective layer. This showed that the modification is happening throughout the entire cross-section, with the 1kPEG diffusing through the permeation pathways and reacting inside them. This bulk modification of the PBI membrane indicates a pore normalisation effect.

The largest pores will have a greater amount of 1kPEG and therefore will have a greater extent of modification. This modification will provide a greater increase in solute resistance inside the larger pores and therefore acts to normalise the pore size. Further evidence that the modification is taking place inside the permeation pathways is provided in **Section 4.3.4**.

The weight difference was also measured between PBI and PBI-DBX, which corresponded to 11.5 g m⁻² weight increase due to crosslinking. This extent of crosslinking was challenged by exploring the solvent stability in a range of solvents. In all cases, there was negligible change in the membrane mass, as shown in **Table 4.3**. This suggests that the degree of crosslinking is sufficient to provide a membrane well suited to a wide range of organic solvent purifications.

Table 4.3 Percentage weight change of crosslinked PBI membranes following immersion in a range of solvents, and acidic or basic conditions for a minimum of two weeks.

PBI weight change (%)						
DMAc	Toluene	MeCN	DMF	Chloroform	20 wt% HCl in water (pH < 0)	20 wt% NaOH in water (pH > 14)
0 ± 1	0 ± 1	0 ± 1	-1 ± 1	0 ± 1	+1 ± 1	0 ± 1

The cross-sectional morphology of the membranes was analysed by SEM, shown in **Fig. 4.4a - f**. No significant changes to the underlying finger-like PBI morphology were observed after the modification reaction, compared to before the modification reaction. This was independent of the grafting reagents which were used and suggests that there the graft modifiers are not causing significant degradation or rearrangement of the PBI structure. Therefore, the performance changes are predicted to be due to the grafting of 1kPEG and 2-MEA, not instability of the PBI membrane in the grafting solutions.

Finally, the topography of the membranes was analysed with AFM. Before modification, **Fig. 4.4g**, the membrane had a maximum topographic peak height of 17.7 nm. Due to the small size of 2-MEA, this maximum height was found to be relatively unchanged after modification with 2-MEA (M-0), **Fig.**

4.4h. However, after modification with a larger molecule (1kPEG), the amount of variation in the peak height, and the maximum peak height were found to be lower, with a maximum height of 14.5 nm, **Fig. 4.4i**. This was because the 1kPEG, which was grafted to the top surface of the membrane, collapsed upon drying for the AFM analysis. This led to a normalisation of the membrane topography and demonstrated that 1kPEG was successfully grafted onto the PBI membrane.

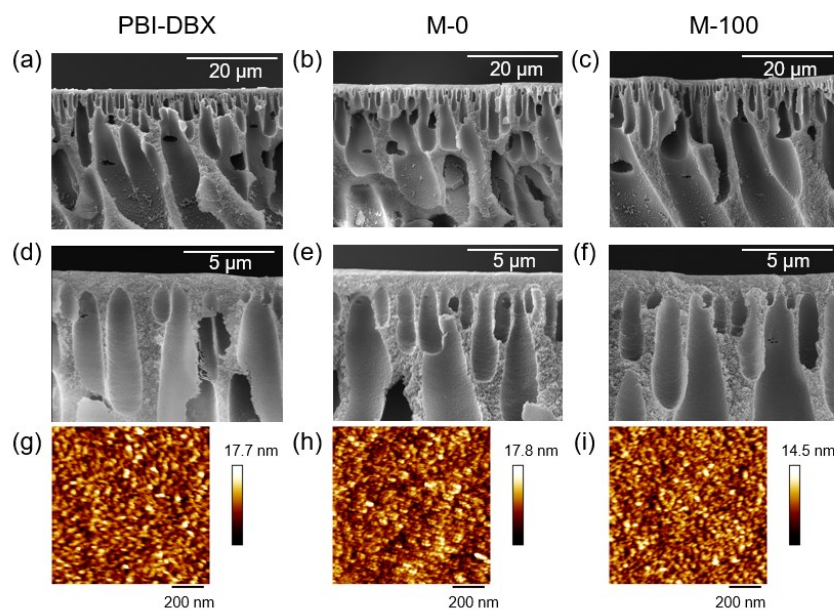


Fig. 4.4 The cross-sectional SEM of (a) PBI-DBX, (b) M-0, (c) M-100, with high resolution SEM for (d) PBI-DBX, (e) M-0, (f) M-100. The top surface AFM of (g) PBI-DBX, (h) M-0, (i) M-100.

4.3.3 Performance

The separation performance of the modified PBI membranes was tested in a range of polar and nonpolar solvents. They were tested with a standard marker series for OSN: different molecular weight PEGs [11]. PEG has the advantage of being uncharged, cheap, and it is possible to simultaneously detect a wide range of different molecular weights. However, using PEG has potential issues with linear polymer reptation [233] and it is not directly representative of many ‘real’ solutes. For membrane performance in a real system, readers are referred to **Chapter 7** and Yeo et al. [99]. The permeance and rejection of the membranes are shown in **Fig. 4.5**.

The permeance in all polar solvents followed the order: M-0 > M-90 > M-97.5 > M-100. This was believed to be due to the increased solvent resistance caused by a large polymer, 1kPEG, in the membrane pores, relative to very little change in resistance from a small molecule, 2-MEA. The greater the extent of 1kPEG modification, the greater the resistance to solvent flow, and therefore the greater decrease of the flux. The molecular weight cut off (MWCO) was also affected by the extent of 1kPEG modification: M-0 > M-90 > M-97.5 > M-100. This was also due to 1kPEG extending into the pores and preventing permeation of large solutes, with a greater extent of modification providing a greater change to the rejection curve. In acetonitrile the MWCO could be controlled between 2,000 g mol⁻¹ and 20,000 g mol⁻¹, depending on the modification conditions. Additionally, the rejection of 35,000 g mol⁻¹ PEG was greater than 99% for all polar solvents, suggesting that defect-free membranes were manufactured.

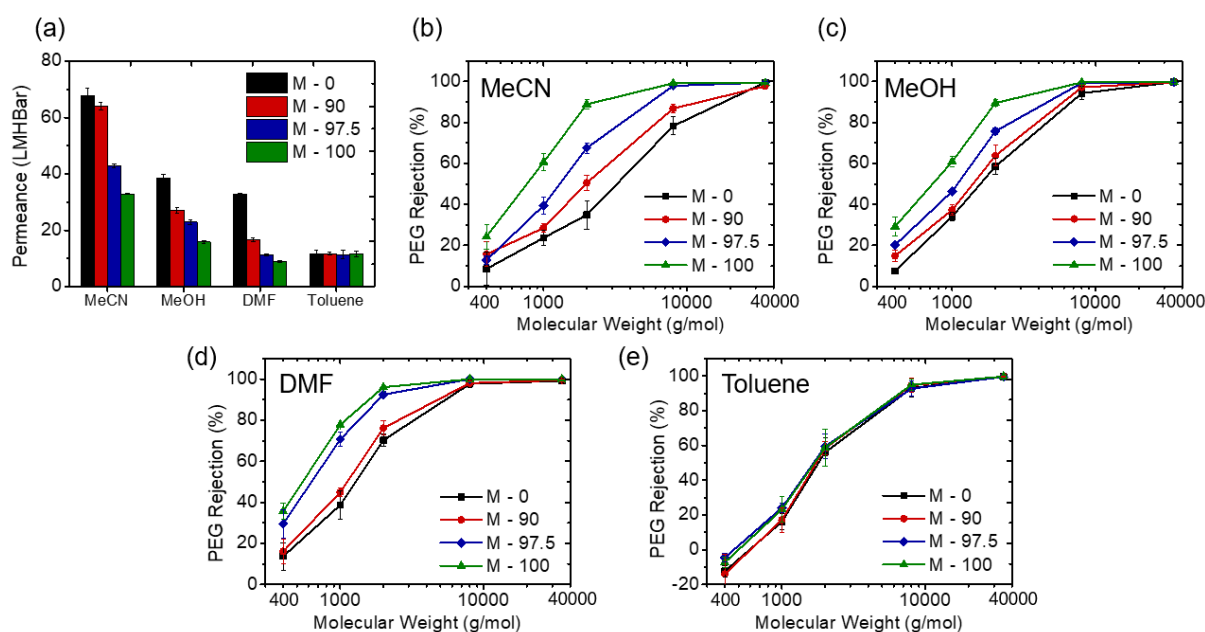


Fig. 4.5 (a) The solvent permeance of the membranes developed for this report were measured at 10 bar, 30 °C, and a cross-flow of 75 L h⁻¹. The rejection profiles were investigated with 1 g L⁻¹ of each PEG: 400, 1000, 2000, 8000, and 35000 g mol⁻¹ in (b) acetonitrile, (c) methanol, (d) DMF, and (e) toluene.

In a purification process, there are two key rejection values. The rejection of the permeating species should be as low as possible, while the rejection of the retained species should be as close to 100% as

possible. For a constant volume diafiltration, a rejection below 40% will allow for 99% removal of the permeating species in approximately eight diafiltration volumes. Therefore, a 40% cut-off was deemed to be the maximum acceptable rejection for any permeating species. Based on this value, these membranes allow for the successful permeation of a molecule between 500 and 2,000 g mol⁻¹, with a MWCO between 2,000 g mol⁻¹ and 20,000 g mol⁻¹ respectively, depending on the extent of 1kPEG modification. This 40% rejection cut-off is ideally suited to a number of DSP syntheses including liquid phase peptide synthesis [99] and oligonucleotide synthesis [7].

Comparison with commercial membranes was difficult due to a lack of membranes with a tight ultrafiltration MWCO that are stable in organic solvent. Of the current commercial OSN membranes, the Duramem, Puramem and Borsig ranges have a maximum MWCO of 900 g mol⁻¹, 600 g mol⁻¹ and 900 g mol⁻¹ respectively, while SolSep membranes, which are stable in strong polar aprotic solvents, have a MWCO of less than 1,000 g mol⁻¹ [11,234,235]. Therefore, the membranes in this work fill a gap in a molecular weight range not currently amenable to organic solvent ultrafiltration.

In non-polar toluene a very different trend was observed. All the membranes still had greater than 99% rejection of 35,000 g mol⁻¹ PEG. However, modification with different coverages of 1kPEG has very little effect on the rejection or the permeance. This is attributed to the molecular conformation of 1kPEG in solution. In polar solvents, the 1kPEG forms a ‘mushroom’ structure, which extends into the pore, ensuring high levels of interaction with the passing solvent and solutes [236]. However, in a nonpolar solvent, the interactions between 1kPEG and the membrane surface may be stronger than the interactions between 1kPEG and the solvent. This causes the 1kPEG to collapse into a ‘pancake’ shape and provide minimal flow resistance [237]. This means that the effect of 1kPEG modification is very small in nonpolar solvents, relative to the effect of the extended 1kPEG in a polar solvent.

4.3.4 Graft Location

In order to investigate the location of grafting within the PBI membranes, two new techniques for modification with 1kPEG were used. These have been compared to the ‘standard modification’ technique which was used in this report to synthesise the membranes in **Section 4.3.2** and **4.3.3**. In the

standard modification technique, the 1kPEG modifying solution is in contact with both sides of the PBI membrane. However, in the first new technique ('top modification'), the 1kPEG modifying solution was exclusively in direct contact with the top selective layer of the PBI membrane. To ensure the modifying solution was not in contact with the underside of the membrane, an impermeable sheet of PP was used to cover the underside membrane, as shown in **Fig. 4.6a**. In the second new technique ('bottom modification'), the modification solution was exclusively in direct contact with the underside of the membrane. In bottom modification, the impermeable PP sheet covered the selective layer of the PBI membrane, shown in **Fig. 4.6b**.

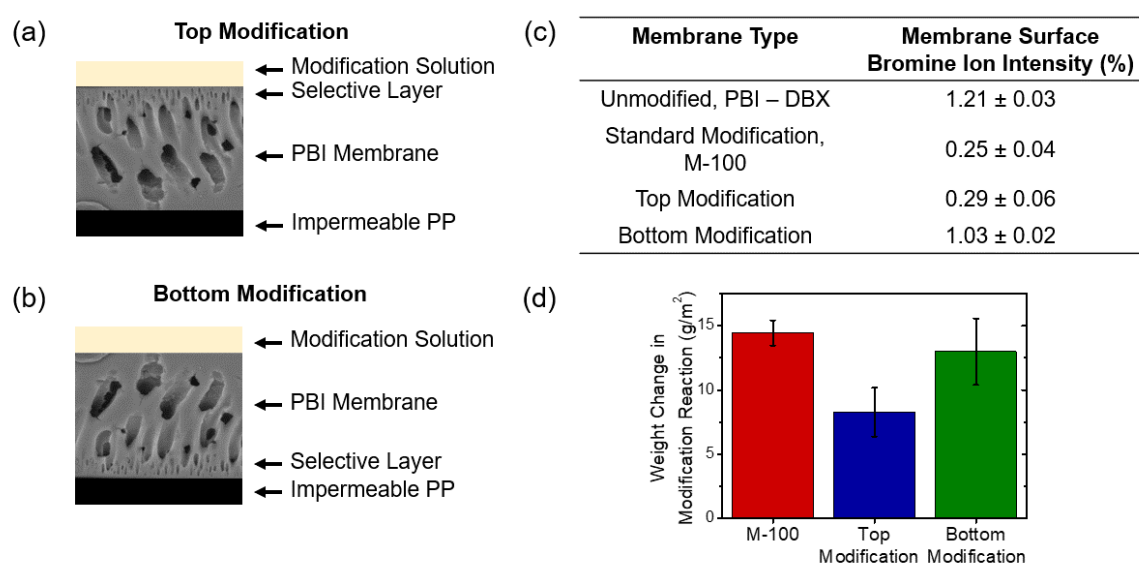


Fig. 4.6 (a) Cross sectional schematic for top modification, showing the selective layer of PBI in contact with the modifying solution. (b) Cross sectional schematic for bottom modification, showing the underside of the membrane in contact with the modification solution. (c) TOF-SIMS quantification of the bromine ion intensity relative to the total ion count for different membrane modification techniques. (d) Specific weight change of each membrane in the modification reaction, relative to PBI-DBX.

TOF-SIMS was used to analyse the bromine ion intensity of the top selective surface layer of the membrane and is quantified in **Fig. 4.6c**. Before modification there was a large intensity of residual bromine on the top surface of the membrane (PBI-DBX). The bromine ion intensity was significantly reduced with both the standard modification technique (M-100) and the top modification technique.

This showed that there were large amounts of 1kPEG grafted to the top surface of the membrane. However, in the bottom modification technique, the bromine ion intensity was similar to the unmodified membrane. This suggests that this technique led to a negligible amount of 1kPEG grafted to the top surface of the membrane.

Fig. 4.6d shows the specific weight change of each membrane in the modification reaction, relative to the unmodified membrane. The top modified membrane had a significantly lower weight increase than M-100. This showed that despite the top surface being fully modified, there was a lower amount of 1kPEG grafted onto the bulk of the PBI. Comparatively, the bottom modified membrane had a similar weight change to M-100. This showed that despite the top surface of the membrane remaining unmodified, the bulk of the PBI was grafted with 1kPEG.

The hypothesis for this is the rate of 1kPEG grafting to the PBI membranes depends on the rate of diffusion of the grafting agent into the membrane. A schematic for this is shown in **Fig. 4.7**. Using the top modification technique, **Fig 4.7a**, the modification solution was in direct contact with the top surface, which allowed for a high extent of modification of the top surface. Below this, there was diffusion of 1kPEG into the permeation pathways in the dense selective layer. This layer was modified first, and to a high extent. However, the modification of this selective layer provided significant hindrance to the 1kPEG diffusing into, and modifying, the bulk of the PBI membrane. As a result, the bulk PBI had a lower extent of modification and therefore the top modified membrane had a lower specific weight change.

In the bottom modification technique, **Fig. 4.7b**, the bulk of the PBI was modified first. Because the bulk PBI has a much more open structure, the diffusion of 1kPEG towards the selective layer was not hindered. Therefore, there was a higher extent of modification in the bulk of the PBI and inside the permeation pathways in the selective layer. This resulted in the bottom modified membranes having a similar weight change to membrane modification from both surfaces (M-100). However, the modification of the selective layer hindered the diffusion of 1kPEG to the top surface of the PBI

membrane. Hence, the very top membrane surface of the membrane remains unmodified and had a high bromine ion count.

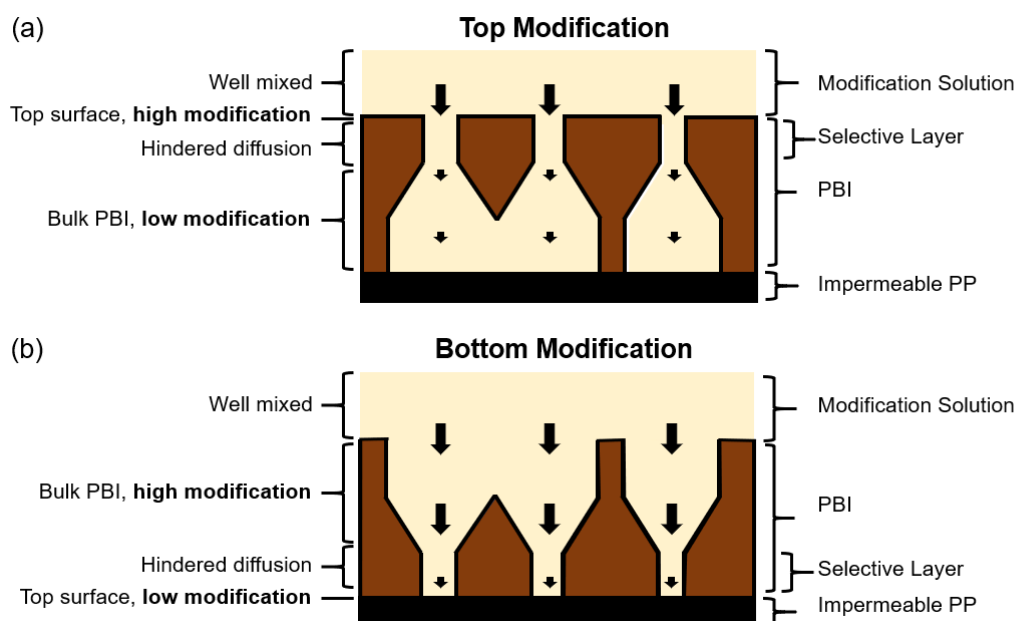


Fig. 4.7 (a) Proposed schematic for top modification. Arrows show the rate of 1kPEG diffusion through the membrane cross section. (b) Proposed schematic for bottom modification.

Fig. 4.8a and **Fig. 4.8b** show the permeance and rejection of each these membranes. The bottom modified membrane, which has 1kPEG grafted exclusively in the permeation pathways but not grafted to the top surface, had a similar permeance and rejection to the M-100 membrane, which has 1kPEG grafted to the inside of the permeation pathways and to the top surface. This showed that it is the grafting of 1kPEG to the inside of the permeation pathways of the membrane, which is controlling the performance, and not the grafting of 1kPEG to the top surface.

The top modified membrane also had similar performance to the M-100 membrane. This suggests that in all cases the key permeation pathways have been saturated with 1kPEG. Therefore, to minimise membrane manufacturing cost, using a top modification methodology could be selected as it requires a lower mass of 1kPEG modifier. However, maximising the mass uptake of 1kPEG, by ensuring that the entirety of every accessible permeation pathway is fully modified, is predicted to give a more consistent

performance. Therefore, the standard modification methodology (M-100), with diffusion of 1kPEG from both the top and bottom surface, was our optimum choice of modification for scale up.

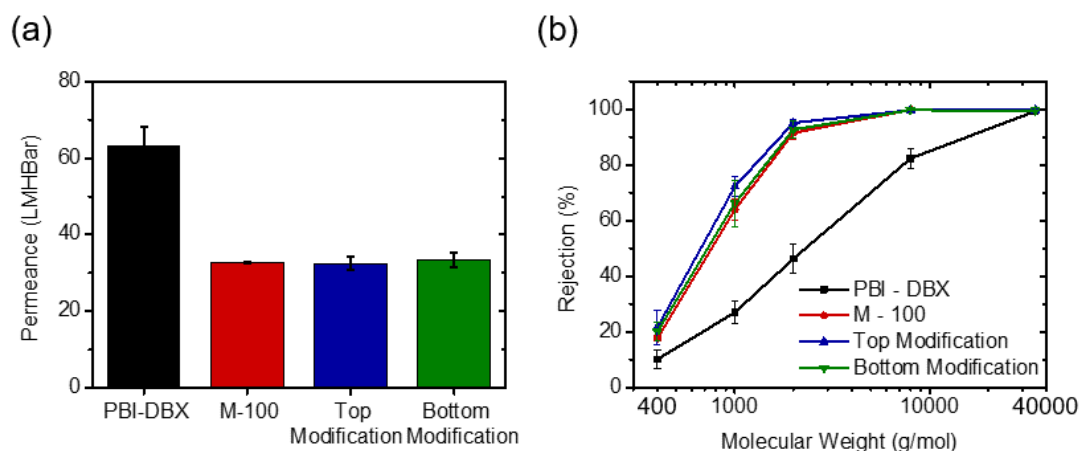


Fig. 4.8 (a) The solvent permeance of the membranes taken in acetonitrile at 10 bar, 30 °C, and a crossflow rate of 75 L h⁻¹. (b) The membrane rejection profiles measured with 1 g L⁻¹ of each PEG: 400, 1000, 2000, 8000, and 35000 g mol⁻¹ in acetonitrile.

4.3.5 Scale-Up

A well-documented challenge in the industrialisation of membrane technology is the difficulty encountered in scaling up a new membrane fabrication approach [238]. Therefore, in order to show the industrial applicability of modified PBI membranes the membrane was continuous cast, crosslinked, and modified at 5 m × 0.3 m scale. To demonstrate reproducibility, this membrane was fabricated into three M-100 spiral wound modules from three different membrane casts with three different modification reactions.

The rejection and flux of the modules and flat sheet membranes in methanol is shown in **Fig. 4.9**. The rejection profile for the modules was similar to that of the flat sheets and shows that it is possible to produce larger quantities of consistent, defect-free membrane by continuous casting. However, the permeance was lower in the spiral wound modules, with a large degree of flux variation between the individual modules. This is believed to be due to difficulties in rolling these small experimental modules, where small deviations in the module glue lines can lead to significant loss of active membrane

surface area. These arise from the current lab scale module production methods. Therefore, it is believed that the technology is scalable and that the discrepancies between modules would be reduced with the production of modules having a larger surface area.

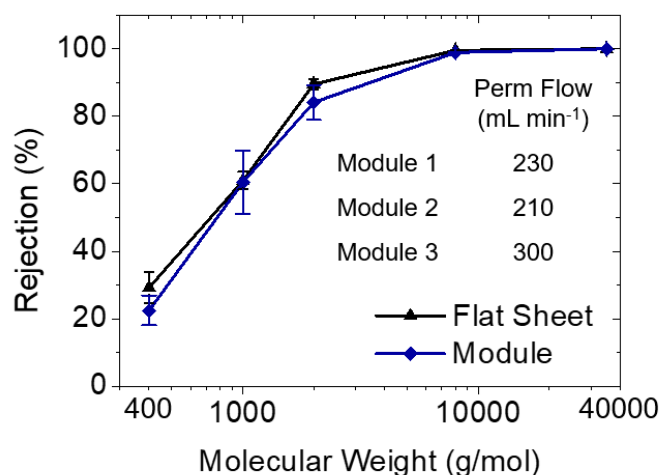


Fig. 4.9 The methanol permeate flow rate and PEG rejection profiles of three spiral wound modules ($\sim 0.1 \text{ m}^2$) of the M-100 membrane developed here compared to flat-sheet membrane (0.0014 m^2), measured at 10 bar, 30°C , and a cross-flow rate of 75 L h^{-1} . The rejection profiles were investigated with 1 g L^{-1} of each PEG: 400, 1000, 2000, 8000, and 35000 g mol^{-1} .

4.4. Conclusions

Solvent stable crosslinked PBI membranes were synthesised and modified with 1kPEG. Competitive reaction with 2-MEA was used to control the extent of 1kPEG modification, and therefore to adjust the membrane performance. In polar solvents, increasing the extent of 1kPEG modification decreased the flux and decreased the molecular weight cut-off from tight ultrafiltration to loose nanofiltration membranes. In a non-polar solvent, the extent of modification had no effect on the rejection or permeance. This technique was scaled up to produce spiral wound modules, which showed similar rejection profiles to their flat sheet equivalents and demonstrate the potential for industrial application of modified PBI membranes. These membranes can be used for separations in strong organic solvents that require permeation of a molecule from 500 to 2,000 g mol^{-1} , while retaining a molecule of 2,000 to 20,000 g mol^{-1} respectively, which commercial membranes cannot currently achieve.

Chapter 5: Improving the long-term process stability of polybenzimidazole membranes in organic solvents: when crosslinking is not enough

Abstract

The importance of polymer molecular weight on the long-term process stability of polybenzimidazole (PBI) membranes in organic solvents was investigated. PBI membranes were manufactured from two different polymer molecular weights: the standard molecular weight of PBI polymer, used almost universally for organic solvent stable membranes ($\sim 27,000 \text{ g mol}^{-1}$), and an untested two-fold higher molecular weight ($\sim 60,000 \text{ g mol}^{-1}$). After crosslinking, all PBI membranes were chemically and mechanically stable. However, the standard molecular weight PBI membranes were not process stable. During continuous long-term pressurised filtration in DMF, the standard molecular weight PBI membranes suffered slow permeance decline and ageing. This was attributed to rearrangement and densification of the crosslinked polymer chains towards an equilibrium state. Increasing the extent of crosslinking did improve the process stability, however, the standard molecular weight membranes all showed permeance decline during long-term testing. Conversely, the high molecular weight PBI membranes, with the same extent of crosslinking, showed constant permeance and excellent process stability, even during long-term accelerated ageing tests in DMF at 120°C . This was attributed to the increased interchain interactions in the high molecular weight polymer, which reduced the rate of chain rearrangement and densification. This work demonstrates the importance of polymer molecular weight for membrane process stability in organic solvents.

Keywords: Ageing, Chain Mobility, Organic Solvent Nanofiltration (OSN), Organic Solvent Ultrafiltration (OSU), Polybenzimidazole (PBI)

Highlights

- Membranes were cast from a standard molecular weight (MW) PBI and a high MW PBI
- These membranes were crosslinked with DBX to different crosslinking extents
- The PBI MW was critically important to the process stability of the membranes
- Only PBI with a high MW and a high crosslinking extent was process stable in DMF
- The standard MW PBI with the same crosslinking extent was not process stable

5.1 Introduction

Polymeric membranes are ubiquitous in nanofiltration and ultrafiltration due to their low manufacturing cost, low manufacturing energy, and formation from solution phase. They are often produced through nonsolvent induced phase separation (NIPS), which separates a polymer solution into a solvent-rich phase and a solvent-poor phase due to interactions with a nonsolvent. This process is scalable and can produce large surface areas of consistent membrane at low cost [201,239–241].

Nanofiltration and ultrafiltration membranes formed through NIPS usually have a dense top surface and an asymmetric morphology. This is due to the relatively fast phase inversion process which solidifies the polymer chains in a non-equilibrium state. This process creates an excess free volume in the membrane matrix, which forms the permeation pathways or pores. These permeation pathways control the permeance and selectivity of the membrane [239,242–244].

Due to the non-equilibrium state of the polymer matrix, polymeric membranes are susceptible to ageing. Unless the polymer chains are fully immobilised, they will spontaneously rearrange towards the lowest energy conformation. This ageing process typically results in the densification of the membrane selective layer, causing the flux and selectivity to decrease. Inevitably, ageing will decrease the operating lifetime of a membrane and increase the cost of the separation process [245–249].

Separations in organic solvents are a field of ever-increasing interest and ageing is a significant difficulty in this field. It is established that the rate of ageing depends on the immediate environment of a membrane, and in organic solvent nanofiltration (OSN), or organic solvent ultrafiltration (OSU), the membranes are continuously exposed to strong solvents and harsh conditions [9,11,250]. This means the polymer chains in the membrane have an increased susceptibility to rearrangement, and therefore polymeric OSN and OSU membranes are highly susceptible to ageing [248,251–255].

To reduce chain mobility in OSN and OSU, crosslinking is often used. Crosslinking uses thermal, radioactive, or chemical methods to introduce ionic, covalent, or hydrogen bonds between adjacent polymer chains. This technique has been used to increase the stability of a wide range of polymer types,

including polyimide (PI), polyacrylonitrile (PAN), and polybenzimidazole (PBI), with the aim of manufacturing membranes which have long-term process stability [151,176,180,188,256].

An alternative approach to reduce the chain mobility of polymers is to increase the molecular weight of the polymer. A higher polymer molecular weight increases the length of the polymer chains, resulting in an increased amount of chain entanglement and interchain interactions. The molecular weight of the polymer also affects the physico-chemical properties such as the glass transition temperature, thermal decomposition temperature, tensile strength, and elastic modulus [257–259]. This makes molecular weight a critically important parameter in polymer science, and therefore in polymeric membrane formation.

However, there have been few studies to date on the effect of the polymer molecular weight on membranes. For polyethersulfone, Zhou et al. used a molecular weight range from 42 kDa to 120 kDa. They showed that increasing the polymer molecular weight led to higher flux and lower rejection of 20 kDa PEG in aqueous systems [260]. For intrinsically microporous polyether ether ketone, Abdulhamid et al. showed that increasing the polymer molecular weight produced a membrane with lower permeance, but there was little significant change to the molecular weight cut off [259]. Similar studies have been made for other categories of OSN membranes including polyvinylidene fluoride (PVDF) [261,262], polysulfone [174], and polyimide [168]. However, these studies all focused on investigating the effect of the molecular weight on the flux and rejection of the membrane, not on the effect on the long-term performance and susceptibility to ageing.

PBI is a promising polymer candidate for OSN and OSU membranes [199,263,264]. In this study two different PBI molecular weights were used. A 0.7 IV standard molecular weight PBI ($\sim 27,000 \text{ g mol}^{-1}$) was used, which is employed almost universally for OSN membranes [12,189,190]. Additionally, a 1.1 IV high molecular weight PBI ($\sim 60,000 \text{ g mol}^{-1}$) was used, demonstrating the first application of a high molecular weight PBI polymer for organic solvent stable membranes. The effect of the casting and crosslinking conditions on the permeance and rejection of the high molecular weight PBI membrane was investigated, and the performance was compared to the standard molecular weight membrane.

After this, both the high and standard molecular weight PBI membranes were crosslinked with a range of different crosslinking extents and the long-term performance of the resulting membranes. For this study, the long-term performance was measured in a mild polar aprotic solvent, acetonitrile, and a strong polar aprotic solvent, dimethylformamide (DMF). The ageing process was then accelerated in DMF by increasing the working temperature to 120 °C, to understand the effect on long-term process stability.

5.2 Materials and Methods

5.2.1 Materials

A custom 18 wt% solution of the standard molecular weight Celazole Polybenzimidazole (18 wt% PBI with 1.5 wt% lithium chloride in N,N-dimethylacetamide (DMAc)) and a custom solution of high molecular weight PBI (1.1 IV PBI dissolved at 18 wt% with 1.5 wt% lithium chloride in DMAc) were purchased from PBI Performance Products, Inc (USA). Non-woven support Novatexx 2471, made of polypropylene (PP), was purchased from Freudenberg Filtration Technologies (UK). HPLC grade acetonitrile (MeCN), propan-2-ol (IPA), and dimethylformamide (DMF) for membrane synthesis and membrane testing were purchased from VWR (UK). DMAc, α,α' -dibromo-*p*-xylene (DBX), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), and PEG (400 g mol⁻¹) for membrane synthesis were purchased from Sigma Aldrich (UK). PEG (400, 1000, 2000, 4000, 8000, and 35000 g mol⁻¹) for membrane testing was purchased from Sigma Aldrich (UK).

5.2.2 Methods

5.2.2.1 Casting

PBI solutions were diluted to the required weight percentage, as specified in **Fig. 5.1b**, with DMAc. The viscosity was measured with a rheometer (MCR 72, Anton Parr) at 20 °C. The membranes were then cast using the method described in **Section 4.2.2.1** at ~10 m length.

5.2.2.2 Crosslinking

The crosslinking reactions were performed with the method described in **Section 4.2.2.2**, with a 0.15 m × 0.4 m membrane roll in a 160 ml cylinder maintained at 80 °C for either 4 h, 24 h, or 48 h. After this, the membrane was immersed in 2.5% (v/v) DBU in acetonitrile for a minimum of 4 h, then washed with

fresh acetonitrile. Following washing, the membranes were preserved with PEG 400 using the method described in **Section 4.2.2.4**.

5.2.2.3 Characterisation

For elemental analysis, specific weight change, and SEM, the methods described in **Section 4.2.2.6** were used, except with the vacuum oven set to 100 mbar.

Samples for FT-IR were dried and measured using a Perkin Elmer Spectrum 100. Scans were measured from 4000 cm^{-1} to 600 cm^{-1} with 2 cm^{-1} resolution. All measurements were averaged over 16 scans.

Young's modulus was measured by atomic force microscopy (AFM). Samples were dried and then measured on a Bruker Dimension Icon using a RTESPA-300 Cantilever (40 N m^{-1}). Samples were measured with a scan rate of 1 Hz and a peak force frequency of 2 kHz.

Gel permeation chromatography (GPC) samples were analysed using an Agilent 1260 GPC coupled with a diode array detector (DAD) and a refractive index detector (RID). Samples were injected onto two PLgel 5 μm Mixed-D columns and eluted with DMF containing 0.1% (w/v) LiBr. A flow rate of 1 ml min^{-1} was used. The temperature of the column and RID detector were both set to 50 $^{\circ}\text{C}$.

The mean flow pore size was measured using a liquid-liquid displacement porometer (LLDP 1500A, Porous Materials Inc.). A mixture of isobutanol-water 1:1 (v/v) was separated into an isobutanol-rich phase and a water-rich phase. The membranes were pre-soaked in the isobutanol-rich phase and the water-rich phase was used as the displacement liquid. The permeation was measured from 100 to 300 psi, at 5 psi intervals, and correlated to the mean flow pore size.

For stability testing, the method described in **Section 4.2.2.7** was used. Stability tests were performed in acetonitrile at 30 $^{\circ}\text{C}$, DMF at 30 $^{\circ}\text{C}$, and DMF at 120 $^{\circ}\text{C}$. After immersion, the membranes were washed, dried, and weighed.

5.2.2.4 Performance Testing

The performance testing was performed on the rig described in **Section 4.2.2.8**. Four membranes of each type were tested. The rejection was measured using 1 g L^{-1} each of PEG 400, 1000, 2000, 4000,

8000, and 35000 g mol⁻¹. The rig was operated at a cross-flow rate of 60 L h⁻¹ per parallel path, 30 °C and 5 bar. Membrane compaction was completed at 20 bar for a minimum of 24 h prior to sampling. This duration was found to be sufficient to produce relatively constant permeance and rejection.

Long-term performance in acetonitrile and DMF were completed in the same rig and the same conditions, except pure solvent was used. The pressure was maintained at 20 bar throughout and the permeance was measured at regular intervals.

The high temperature DMF permeation experiments were completed in a different setup. The tests were completed in a high temperature rig, as described by da Silva Burgal et al. [265]. Two 51 cm² stirred cross flow cells were placed in series. Each cell had a temperature probe inside the cell connected to a heating and stirrer plate underneath the cell, operated at 750 rpm, 120 °C. A HPLC pump (Gilson 305, 100 mL min⁻¹) provided flow from a feed flask to the membrane cells. The feed flask was placed in a heated oil bath and also maintained at 120 °C. Above 120 °C, the PP backing started to show degradation. The pressure was maintained with a back-pressure regulator and the permeate was returned to the feed flask. The system was maintained at a pressure of 5 bar, under an argon atmosphere. A minimum of two cells from two separate crosslinking reactions were tested, with the rejection and permeance taken every 24 h for 168 h.

5.3 Results and Discussion

5.3.1 Casting and Crosslinking

In this work, two different molecular weights of PBI polymer were used. The first was the standard molecular weight PBI, sold by PBI Performance Products, and commonly used to manufacture membranes for OSN [154,189,266]. This standard molecular weight PBI has an average molecular weight of approximately 27,000 g mol⁻¹. A second higher molecular weight PBI polymer was also used in this work, which is untested for OSN. This polymer had a two-fold higher molecular weight of approximately 60,000 g mol⁻¹. The GPC of both polymers is shown in **Fig. 5.1a**.

These two polymers were continuously cast using NIPS. The standard molecular weight polymer was cast at a dope concentration which is widely reported across literature, 18 wt% PBI in DMAc [12,263].

This membrane was named M0, shown in **Fig. 5.1b**. However, for the high molecular weight PBI, literature is not available on the casting conditions for OSN. Therefore, a range of different polymer concentrations were dissolved in DMAc and used to investigate the parameter space for continuous casting. Three casting concentrations were used: 14 wt%, 16 wt%, and 18 wt%, which were named M14, M16, and M18 respectively. These casting concentrations were selected so that there could be a direct comparison between membranes cast at the same concentration but of different molecular weight, M0 and M18, a direct comparison between membranes cast at a similar viscosity but of different molecular weight, M0 and M14, and an intermediate casting concentration, M16.

Following continuous casting, the specific dry weight of the membranes was measured, shown in **Fig. 5.1b**. All membranes were cast with a consistent knife gap, so the specific dry weight was proportional to the concentration of PBI in the dope solution. Therefore, increasing the dope concentration increased the specific dry weight of the high molecular weight PBI membranes.

For the standard molecular weight PBI, the specific dry weight was slightly higher than the dry weight of the high molecular weight polymer at an equivalent dope concentration. Although the knife height was consistent, the lower viscosity of the standard molecular weight PBI enabled a small amount of additional polymer solution to flow underneath the casting knife, therefore producing a membrane with a higher specific dry weight.

The mean pore size of the different membrane casts was measured using liquid-liquid displacement porometry, shown in **Fig. 5.1b**. For the high molecular weight PBI, increasing the PBI concentration in the dope solution decreased the mean pore size, $M14 > M16 > M18$. This is because, in NIPS, the top surface of the dope solution rapidly precipitates when immersed in the nonsolvent. This is due to the fast initial exchange of solvent and nonsolvent, which forms the characteristic dense top layer and the asymmetric structure of the membrane. This dense top layer is often the controlling factor which determines the pore size [267]. Increasing the concentration of PBI in the dope solution increased the polymer concentration at the nonsolvent interface and also increased the dope viscosity, shown in **Fig. 5.1b**. The higher viscosity decreased the rate of solvent-nonsolvent exchange at the dope-nonsolvent

interface. This resulted in slower phase inversion and led to a more densely packed skin layer with a smaller membrane pore size. Conversely, a low polymer dope composition led to fast solvent-nonsolvent exchange and a membrane with a larger mean pore size. This shows that the pore size of the high molecular weight PBI membranes can be effectively controlled by changing the dope composition, similar to several other polymer types [133,173].

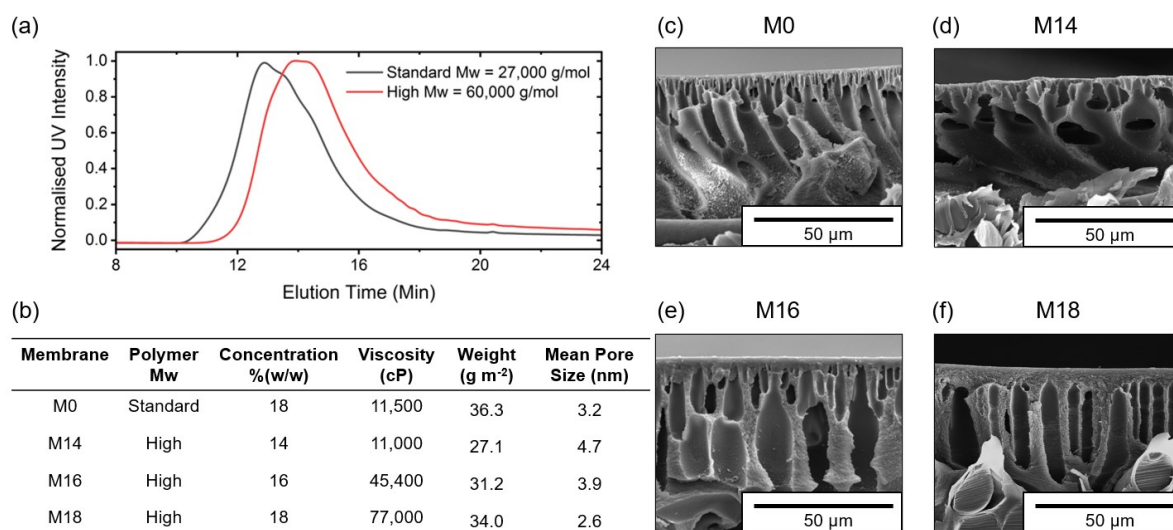


Fig. 5.1 (a) GPC of the standard and high molecular weight PBI used in this work. (b) Details of the membranes manufactured from this work including their casting conditions, casting viscosity, dry weight, and mean pore size, measured using liquid-liquid displacement porometry. The SEM cross sections of each membrane (c) M0, (d) M14, (e) M16, (f) M18.

The phase inversion process was also studied by investigating the cross-section SEM images of the phase inverted membranes, shown in **Fig. 5.1d-f**. During phase inversion, contact of the dope with the nonsolvent causes liquid-liquid demixing. This demixing is often characterised as instantaneous demixing or delayed demixing. Increasing the polymer concentration made the dope solution thermodynamically less stable and a lower concentration of water was required to initiate phase inversion [174,268]. Additionally, increasing the concentration of the dope solution increased the viscosity. For PBI, the higher dope concentration resulted in slower phase inversion and more delayed

demixing. This led to the nucleation of fewer macrovoids at high dope concentrations, and the nucleation of these macrovoids occurred further from the top membrane surface [133,269–271].

Decreasing the polymer molecular weight, to the standard polymer molecular weight, M0, increased the pore size relative to the high molecular weight polymer at the same concentration, M18, shown in **Fig. 5.1b**. The lower polymer molecular weight has lower intermolecular interactions and chain entanglement, which resulted in a lower viscosity of the dope solution. The lower viscosity increased the rate of solvent-nonsolvent exchange upon immersion in the nonsolvent, which resulted in a larger pore size for the M0 membrane than the M18 membrane. Additionally, decreasing the polymer molecular weight increased the thermodynamic stability of the system but increased the rate of polymer chain motion [260]. This favoured instantaneous demixing in the standard molecular weight polymer and resulted in the formation of a thin top dense layer and nucleated a large number of finger-like structures in the sublayer, shown in **Fig. 5.1c**. These finger-like structures were thinner and had a significantly lower wall thickness than the high molecular weight PBI polymer [173,260,270].

After casting, the four membrane casts were all crosslinked. A previously established crosslinking procedure for PBI was used, which has been successfully utilised to crosslink the standard molecular weight PBI [12]. The proposed crosslinking mechanism is shown in **Fig. 5.2a**. In this process, the bifunctional crosslinker, DBX, reacts with two benzimidazole rings and covalently joins the two adjacent polymer chains. This results in a membrane which is chemically stable in acids, bases, and strong organic solvents such as DMF and DMAc [263]. In this work, the crosslinking reaction was stopped after 4 h, 24 h, or 48 h to vary the extent of crosslinking.

Fig. 5.2b shows the percentage weight change of the PBI membrane during the crosslinking reaction. Increasing the crosslinking time increased the specific weight of the membrane due to the incorporation of the DBX into the PBI membrane matrix and shows that the crosslinking was successful. Interestingly, this weight increase was relatively independent from the membrane cast, which shows that crosslinking different casts for the same length of time resulted in a similar extent of crosslinking. During crosslinking, the dry weight of all membrane casts after 24 h crosslinking was increased by $32\% \pm 2\%$,

relative to the initial PBI weight. However, crosslinking for a further 24 h only resulted in a small weight increase, to $37\% \pm 2\%$ of the initial PBI weight after 48 h crosslinking. This suggests that the reaction was close to the maximum achievable extent of crosslinking at these conditions after 24 h.

To demonstrate that the weight increase was due to the reaction of the PBI with the crosslinker, the carbon to nitrogen (C:N) ratio of the membranes was measured, shown in **Fig. 5.2c**. For all membranes, the C:N ratio increased as the crosslinking time increased. This is due to the high carbon content of the DBX crosslinker, which causes the C:N ratio to increase when the DBX is successfully incorporated into the membrane. The rate of change of the C:N ratio was closely correlated to the rate of weight change and corroborates that the crosslinking with DBX was successful.

The successful crosslinking was further demonstrated by measuring the FT-IR of the uncrosslinked and crosslinked M18 membranes, shown in **Fig. 5.2d**. The broad peak between 2500 cm^{-1} and 3600 cm^{-1} represents the N-H stretch of the imidazole ring. Increasing the crosslinking time resulted in a shift in the maximum intensity of this peak from 3200 cm^{-1} to 3400 cm^{-1} , due to a change in the aromatic N-H environment. Additionally, the aromatic C-H peak of PBI at 690 cm^{-1} shifted to 700 cm^{-1} , due to N-substitution during crosslinking. These changes show the changes in the aromatic structure of PBI due to successful crosslinking with DBX [196,272]. Finally, the height of the peak at 1423 cm^{-1} increased with increasing crosslinking time. This corresponds to a C-H alkyl peak, which is only present in the DBX crosslinker, and confirms that the crosslinking was successful.

The actual extent of crosslinking was calculated using the weight change during crosslinking, and the residual bromine content remaining in the membrane matrix after crosslinking, shown in **Fig. 5.2e**. For this calculation, it was assumed that all residual bromine after crosslinking was due to the reaction of only one bromine on the bifunctional crosslinker, thereby leaving a residual bromine in the PBI membrane matrix. Additionally, it was assumed that each PBI monomer had two available sites for crosslinking. From these calculations, it was shown that after 4 h, the total extent of crosslinking was between 19% and 24%, after 24 h the total extent of crosslinking between 36% and 38%, and after 48 h the total extent of crosslinking was between 40% and 42%.

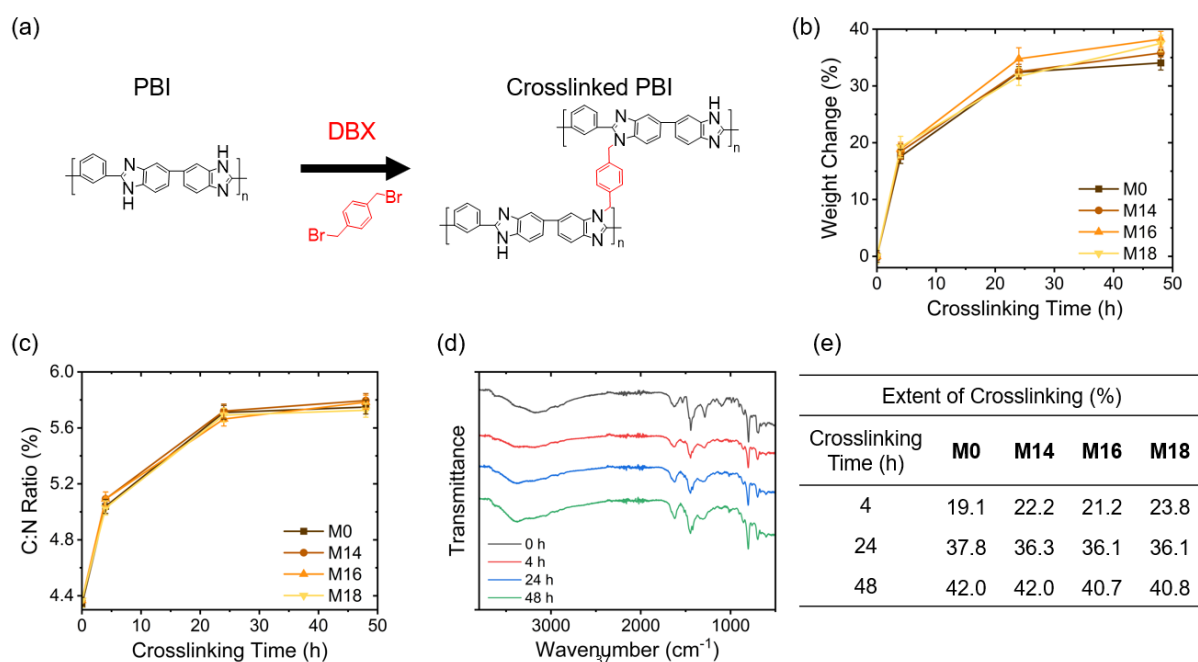


Fig. 5.2 (a) The proposed reaction scheme for the crosslinking of PBI [12]. (b) The weight change during the crosslinking reaction of the four PBI membrane casts. (c) The carbon:nitrogen mass ratio during the crosslinking reaction of the four PBI membrane casts. (d) The FTIR of the M18 membrane after 0 h, 4 h, 24 h, and 48 h of crosslinking. (e) The calculated extent of crosslinking for each membrane.

These calculations showed that after 24 h a significant proportion of uncrosslinked monomers remained. However, increasing the crosslinking time did not significantly improve the extent of crosslinking. This is predicted to be due to the structure of the solid membrane matrix which results in some of the PBI monomers being inaccessible to the bulky DBX crosslinker. This limits the maximum extent of achievable crosslinking, and results in the maximum extent of achievable crosslinking being significantly lower than the maximum theoretical extent of crosslinking. Additionally, after a fixed crosslinking time, the extent of crosslinking was similar for the four membrane casts. This confirmed that for the membranes which were manufactured in this work, the rate of crosslinking was relatively independent from the membrane cast.

5.3.2 Membrane Performance

The performance of these membranes for OSU was investigated in DMF, shown in **Fig. 5.3**. The membranes crosslinked for 4 h, 24 h, and 48 h were solvent stable, and the performance of all membrane casts were investigated. The uncrosslinked membranes were not chemically stable in DMF so the performance of these membranes could not be measured.

The rejection of each membrane was measured in DMF using a series of PEG molecular weights as markers, shown in **Fig. 5.3a-d**. For the different casts, the trend of the molecular weight cut off (MWCO) was correlated closely to the trends in the mean flow pore size of the uncrosslinked membranes, shown in **Fig. 5.1b**, which were evaluated in **Section 5.3.1**. Comparing the different polymer molecular weights at an equivalent dope composition, the high molecular weight membrane had a lower MWCO than the standard molecular weight membrane, $M18 < M0$, due to the slower rate of phase inversion of the high molecular weight polymer. For the high molecular weight membranes, decreasing the concentration of the dope solution increased the MWCO, $M18 < M16 < M14$, due to the increased rate of phase inversion as the dope concentration was decreased.

These rejection trends were closely correlated with the mean flow pore size of the uncrosslinked membranes because the MWCO was relatively independent from the extent of crosslinking. For M0, increasing the duration of the crosslinking reaction increased the extent of crosslinking from ~19.1% to ~42%. However, the MWCO was approximately 4000 g mol^{-1} for all crosslinking extents. This was consistent across all four casts and shows that the PBI membranes were highly stable throughout the crosslinking process, with the pore size not significantly changed during crosslinking.

Compared to the rejection profiles, the permeance with different crosslinking extents showed a significantly different trend. Although the rejection curve was relatively independent of the crosslinking extent, the permeance increased with increasing crosslinking extent, shown in **Fig. 5.3e**. For the M0 membrane, this corresponded to 26.4, 29.6, and 30.1 LMHBar for 4 h, 24 h, and 48 h crosslinking time respectively. The increased permeance was attributed to a change of the hydrophilicity of PBI membrane. The water contact angle of the M0 membrane decreased from 72° for the uncrosslinked

membrane to 55° for the 48 h crosslinked membrane. Therefore, increasing the crosslinking time increased the membrane hydrophilicity, which results in an increased permeance for polar solvents [199].

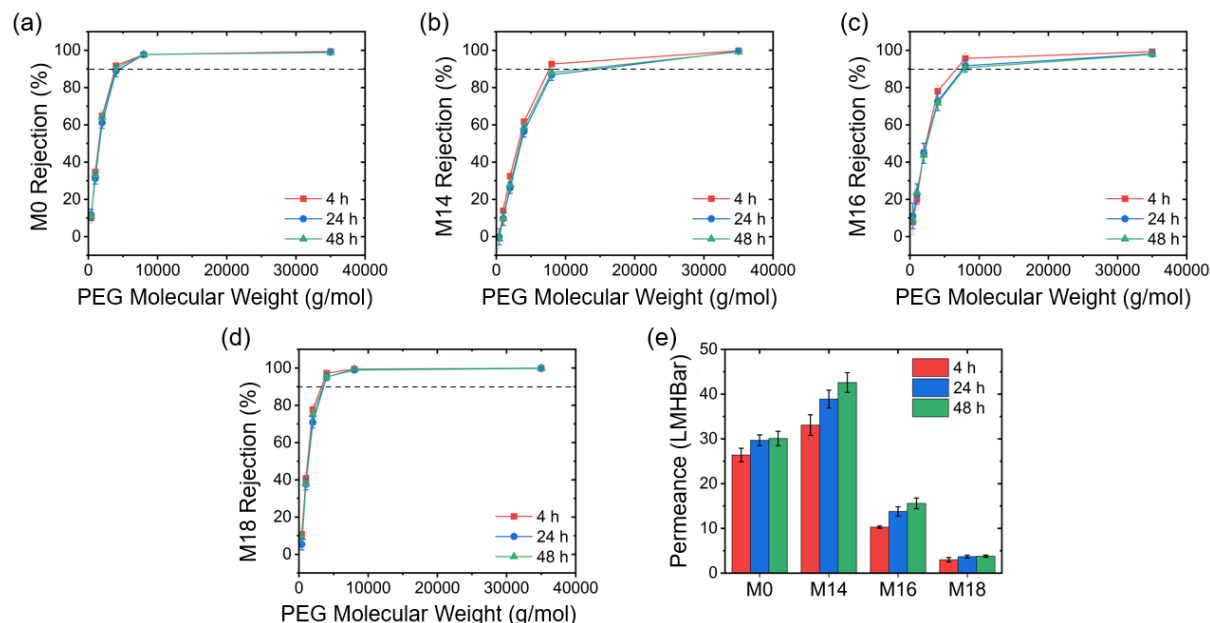


Fig. 5.3 The rejection of the 4 h, 24 h, and 48 h crosslinked PBI membranes for (a) M0, (b) M14, (c) M16, (d) M18. The rejection was measured after compaction with 1 g L^{-1} each of PEG 400, 1000, 2000, 4000, 8000, 35000 g mol^{-1} in DMF at 5 bar, 60 L h^{-1} , 30°C . (e) The permeance of membranes M0 to M18.

Comparing the different membrane casts, the permeance followed the trend $\text{M14} > \text{M0} > \text{M16} > \text{M18}$ for all crosslinking extents. The permeance trend of the high molecular weight membranes followed the same trend as the rate of phase inversion and the membrane pore size, $\text{M14} > \text{M16} > \text{M18}$. However, the standard molecular weight PBI membrane had a significantly higher permeance than expected, based on the MWCO. The M0 membrane had a similar MWCO to the high molecular weight PBI manufactured at the same dope concentration, M18. However, the permeance of the standard molecular weight membrane was ~ 10 -fold higher, corresponding to a permeance between the M14 and M16 membranes. This was due to the low viscosity of the standard molecular weight polymer which resulted in fast solvent-nonsolvent exchange and delayed demixing to form a very thin dense top surface on top

of a finger-like sublayer, similar to M14. However, the higher thermodynamic stability, lower chain entanglement, and lower interchain interactions resulted in a smaller pore size than M14. This showed the benefits of the standard molecular weight polymer, which can be used to manufacture membranes with significantly better permeance for a chosen selectivity.

In terms of industrial applications of these membranes, the important rejection values for organic solvent separation membranes are the MWCO, and the rejection of a molecule which can permeate through the membranes. For a constant volume separation of two species, one of the most common applications of OSN and OSU, a species can be effectively removed in eight system volumes if it has less than 40% rejection. Thus, the molecular weight of a molecule with 40% rejection is an important parameter, referred to here as the MW40. For the membranes in this work, the MW40 was between 1000 g mol^{-1} and 2500 g mol^{-1} . With this MWCO and MW40, these membranes are highly effective for the separation of molecules up to 2500 g mol^{-1} , from molecules larger than 8000 g mol^{-1} , which current commercial membranes cannot achieve [263]. This makes them ideal for a number of applications, including support materials for OSN, catalyst recovery, degumming, and the iterative synthesis of peptides, oligonucleotides, and sequence defined polyethers [29,99,135,137,273,274].

5.3.3 Susceptibility of Crosslinked Membranes to Densification

Most membranes prepared by NIPS are either stored wet or stored with pore preserving agents. This is because the drying of porous membrane results in large capillary forces in the membrane, which cause the porous structure of NIPS membranes to collapse and densify. This results in the irreversible loss of the membrane permeance [275]. However, if the membranes are crosslinked, they should have decreased susceptibility to pore collapse and densification. To test the susceptibility of crosslinked PBI to structural changes and pore collapse, the membranes were dried without pore preservation agents and the permeance was tested in acetonitrile, shown in **Fig. 5.4**.

All membranes showed a significantly lower permeance after drying without pore preserving agents. For the uncrosslinked membranes, drying without pore preserving agents reduced the permeance of all membrane casts by close to 100%. However, increasing the extent of crosslinking increased the flux

retention after drying. This is because the covalent crosslinking fixes the polymer chains in place with greater strength. This results in stronger interchain interactions and requires a higher capillary force to collapse the membrane pores and densify the crosslinked membrane [265,275]. Therefore, increasing the extent of crosslinking resulted in higher flux retention when the membranes were dried without pore preserving agents.

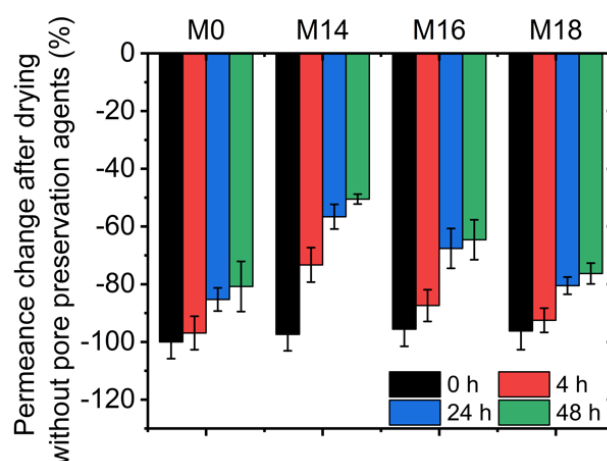


Fig. 5.4 The permeance change of the 0 h, 4 h, 24 h, and 48 h crosslinked membranes after drying without a pore preservation agent compared to drying with a pore preservation agent. The permeance was measured in acetonitrile at 5 bar, 60 L h^{-1} cross flow, 30°C , after 24 h compaction.

Comparing the different high molecular weight casts, the flux loss of the 48h crosslinked membranes followed the trend $M14 < M16 < M18$. This is because the capillary forces during drying are inversely proportional to the pore size of the membrane. Hence, the flux loss on drying of the high molecular weight crosslinked membranes was inversely proportional to the membrane pore size and rate of phase inversion [276]. Conversely, the standard molecular weight membrane, M0, did not fit with this relationship. The 48 h crosslinked M0 membrane had a larger pore size than the 48 h crosslinked M18 membrane. However, the M0 membrane also had a higher flux loss when dried. This suggests that the standard molecular weight membranes are more susceptible to pore collapse and densification than the high molecular weight membranes.

The permeance loss of the PBI membranes when dried shows that if the force deforming the membranes is sufficiently high, the membranes are susceptible to polymer chain rearrangement and densification of the membrane selective layer. The extent of rearrangement was reduced by increasing the extent of crosslinking. However, even after 48 h crosslinking of PBI with DBX, a high enough crosslinking extent was not achieved which stopped all flux loss on drying. Therefore, the crosslinked polymer chains are still susceptible to motion, and if these membranes are used in strong organic solvents for long durations, the DBX crosslinked PBI may be susceptible to pore collapse and ageing.

5.3.4 Long-Term Stability of PBI Membranes

The long-term chemical and process stability of the PBI membranes was investigated in a mild polar solvent, acetonitrile, and a strong polar solvent, DMF [8].

First, the chemical stability of the PBI membranes was investigated in acetonitrile, shown in **Fig. 5.5a**. This was determined by measuring the weight change after four-weeks immersed in acetonitrile. The uncrosslinked and crosslinked PBI were all chemically stable in acetonitrile, with a maximum weight loss of 2% ($\pm 2\%$).

The process stability of these membranes was then investigated by measuring the permeance over 168 h continuous cross-flow testing, shown in **Fig. 5.5b-e**. For all membranes, there was a significant permeance decline for the first 24 h. This was due to membrane compaction. In this work a high pressure was used to ensure the compaction was fast and also simulate the mechanical effect of longer-term performance at a lower operating pressure. This high pressure resulted in a high initial extent of compaction, but the compaction appeared complete after 24 h. The percentage permeance loss due to compaction was more significant for the membranes manufactured from a lower polymer concentration, $M14 > M0 > M16 > M18$. This is attributed to the greater percentage of macrovoids and lower specific dry weight of the M14 membrane, which makes the membrane more susceptible to changing performance with pressure [277]. However, even after significant compaction, the M14 membrane still showed a high permeance.

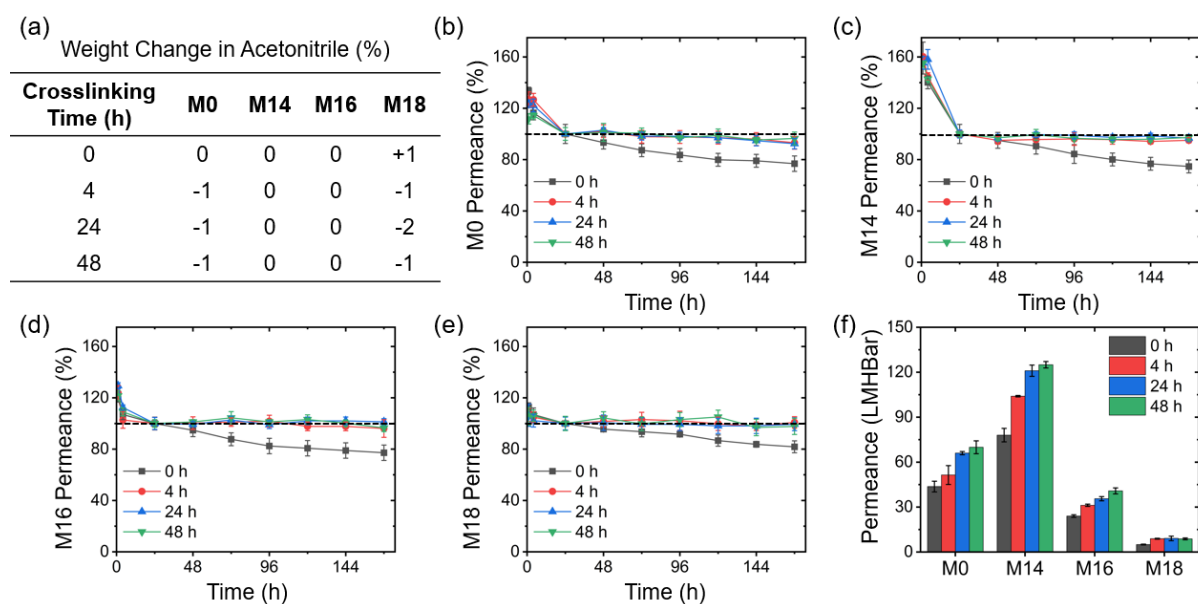


Fig. 5.5 (a) The chemical stability of the PBI membranes, shown by the weight change after soaking in acetonitrile for a minimum of four weeks. (b) The long-term permeance of PBI membranes crosslinked for 0h, 4 h, 24 h, and 48 h relative to the permeance after 24 h of M0, (c) M14, (d) M16, (e) M18. The permeance was measured in acetonitrile at 20 bar, 60 L h^{-1} , 30°C . (f) The permeance of the PBI membranes in acetonitrile after 24 h compaction.

Due to the variable permeance for different crosslinking times, and the variable membrane compaction, the permeance was normalised to the permeance after 24 h, shown in **Fig. 5.5f**. The permeance after 24 h compaction in acetonitrile showed the same trends as the permeance in DMF, described in **Section 5.3.2**. Similar to DMF, decreasing the dope concentration resulted in an increase in the permeance, and the permeance of the M0 membrane was between the permanence of M14 and M16. Additionally, for each cast, the permeance was lowest for the uncrosslinked membranes, and the permeance increased as the crosslinking time was increased.

During long term testing, the crosslinked membranes from all four casts showed less than 8% flux decline between 24 h and 168 h. This showed the excellent process stability of the crosslinked PBI membranes in acetonitrile. Conversely, the uncrosslinked membranes suffered a significant loss of permeance over the same duration. This permeance decline corresponded to between 19% and 26% for all membrane casts and showed that the uncrosslinked membranes were not process stable.

The process instability of the uncrosslinked membranes could be due to multiple reasons. The first potential reason is chemical instability. Here, a chemically stable membrane is defined as a membrane which shows no dissolution or weight loss upon immersion in a specific solvent for a prolonged time period. In the soak test the uncrosslinked membranes were chemically stable in acetonitrile over a four-week period. Therefore, chemical instability is unlikely to be the cause of the permeance decline.

The second potential reason process instability is mechanical instability. This study investigated the performance of the membranes under continuous pressure and the uncrosslinked membrane may not have been mechanically stable at this pressure. To investigate the mechanical stability, the Young's modulus was measured by AFM. During crosslinking the Young's modulus increased. The Young's modulus of the M0 membrane was 2.3 GPa after 0 h crosslinking, and 2.7 GPa and 48 h crosslinking. However, the Young's modulus for the uncrosslinked M14 membrane was 3.2 GPa. Thus, the uncrosslinked M14 membrane, which showed permeance decline, had a higher Young's modulus than the 48 h crosslinked M0 membrane, which showed no permeance decline. The mechanical stability is discussed further in **Section 5.3.5**.

The third potential reason for the loss of permeance is due to the rearrangement of the polymer chains and densification of the membrane. In dry conditions, densification of bulky membranes does not usually occur in the time frame of lab experiments [278]. However, in acetonitrile, the rate of chain rearrangement and pore collapse will be different. Although acetonitrile does not have a high enough solvent power to fully dissolve the uncrosslinked PBI, acetonitrile may promote mobility of the polymer chains, and plasticise the PBI [249,279]. This enables the membrane to rearrange towards a more stable equilibrium position, leading to densification of the selective layer and permeance decline. Due to the permeance decline only occurring in the uncrosslinked PBI, chain rearrangement is the most likely cause of the process instability. This is because, after crosslinking, the polymer chains are strongly held in position by the covalent crosslinker. This prevents chain mobility and results in no permeance decline of the crosslinked membranes in acetonitrile.

Of particular note from the process stability tests is the effect of membrane pore size on the permeance decline of the high molecular weight uncrosslinked membranes. Increasing the membrane pore size led to a more significant permeance decline of the uncrosslinked membranes, corresponding to 19%, 23%, and 26% for the M18, M16, and M14 membranes respectively. The rate of permeance decline is hypothesised to be proportional to the pore size because of the faster rate of phase inversion of the membranes with a larger pore size. This solidifies the polymer chains further from their equilibrium position and makes them more susceptible to rearrangement and densification.

The long-term chemical stability of the PBI membranes was also evaluated in DMF. DMF is a stronger solvent than acetonitrile for PBI, and the uncrosslinked PBI was observed to visually dissolve in DMF within 24 h. The weight change for the uncrosslinked membranes soaked in DMF for four weeks was between -99% and -102% ($\pm 2\%$), which showed that these membranes were chemically unstable, shown in **Fig. 5.6a**. However, after four or more hours of crosslinking, the membranes were fully solvent stable, with a maximum weight change of 2 % ($\pm 2\%$) in the four-week soak tests.

The process stability of the 4h, 24h, and 48 h crosslinked membranes were then investigated by measuring the permeance over 168 h continuous cross-flow testing in DMF, shown in **Fig. 5.6b-e**. For the first 24 h, all membranes showed significant compaction in DMF. Due to this compaction, the permeance was normalised to the permeance after 24 h, shown in **Fig. 5.3e**. The extent of compaction showed the same trend as the compaction in acetonitrile with the highest compaction in $M14 > M0 > M16 > M18$. The percentage permanence decline in DMF due to compaction was slightly higher than in acetonitrile but occurred over a similar time-period of 24 h.

For the standard molecular weight membrane, M0, all crosslinking extents showed process instability over 168 h continuous filtration. The process instability was highest in the 4 h standard molecular weight crosslinked PBI, which showed 29% flux decline between 24 h and 168 h. Increasing the extent of crosslinking resulted in a lower permanence decline. However, even with the higher extent of crosslinking, the membranes were not process stable, with 19% and 17% permeance decline for the 24 h and 48 h crosslinked membranes respectively. Therefore, with the extent of DBX crosslinking used

in this work, the standard molecular weight PBI membranes were not process stable and cannot be used for any long-term or industrial separations in DMF.

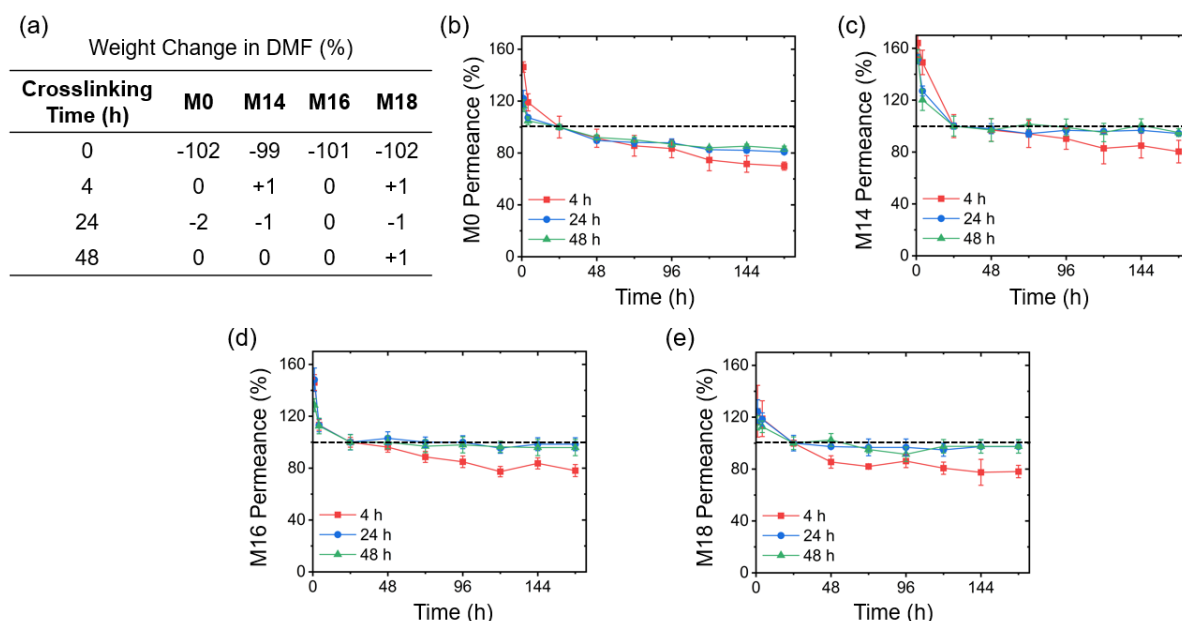


Fig. 5.6 (a) The chemical stability of the PBI membranes shown by the weight change after soaking in DMF for a minimum of four weeks. (b) The long-term permeance of PBI membranes crosslinked for 4 h, 24 h, and 48 h relative to the permeance after 24 h of M0, (c) M14, (d) M16, (e) M18. The permeance was measured in DMF at 20 bar, 60 L h^{-1} , 30°C .

Similar to the process instability in acetonitrile, the process instability in DMF cannot be due to chemical instability as the crosslinked M0 membranes showed no weight change in DMF soak tests. Similarly, this cannot be due to purely mechanical instability. The operating pressure used to determine the long-term performance in DMF was the same as the operating pressure used to determine the long-term performance in acetonitrile. In acetonitrile, the 4 h, 24 h, and 48 h crosslinked M0 membranes were process stable and showed no permeance decline, so these membranes are mechanically stable at this operating pressure. Therefore, the lack of process stability in DMF is again attributed to the rearrangement of the polymer chains.

Although the crosslinked polymer chains should be held in position by the covalent crosslinkers, it was shown in **Section 5.3.3** that the crosslinked polymers are still susceptible to rearrangement and

densification. Additionally, the rearrangement of crosslinked polymer membranes in organic solvents has been previously described, which has resulted in significant densification and permeance decline [265]. This rearrangement has been shown to be highly dependent on the process conditions and process solvent. However, good solvents for the polymer matrix, such as DMF, have been shown to lead to fast densification of polymeric membranes [184].

Due to the solvent strength of DMF, the rate of PBI rearrangement was fast, relative to the rate of PBI rearrangement acetonitrile. This led to the membrane densifying faster and resulted in a significant reduction in the membrane permeance over the 168 h testing period. If the extent of crosslinking could be further increased, it might be possible to make the standard molecular weight membrane process stable in DMF. However, from the calculated crosslinking extent in **Fig. 5.2e**, increasing the crosslinking time had a diminishing increase on the extent of crosslinking. Therefore, significantly different crosslinking conditions, or a different crosslinker are required to make the standard molecular weight PBI membrane process stable in DMF.

For the high molecular weight membranes, the 4 h crosslinked PBI membranes also showed process instability. However, the 24 h and 48 h membranes were process stable, with less than 5% flux decline over 168 h. These membranes have a similar extent of crosslinking to the 24 h and 48 h crosslinked standard molecular weight PBI membranes. Therefore, the improved process stability is predicted to be due to the increase in the polymer molecular weight. A higher molecular weight polymer has increased interchain interactions and increased chain entanglement, which is shown by the increased viscosity of the dope solution [280,281]. The interchain interactions and chain entanglement strongly hold the polymer chains in position within the membrane matrix. Therefore, the higher molecular weight polymer requires a higher force to rearrange the polymer chains. This results in a membrane which is less susceptible to densification and, therefore, has higher process stability. This effect of polymer molecular weight, and the presence of a critical polymer molecular weight, on polymer ageing is well established [282,283]. However, this is the first demonstration of the importance of polymer molecular weight on crosslinked membrane ageing in organic solvent separations.

For the 4 h crosslinked high molecular weight PBI membrane, the combination of low crosslinking extent and high molecular weight does not produce a high enough chain stability to fully immobilise the polymer chains. However, the 24 h and 48 h crosslinked membranes have a higher crosslinking extent and a high polymer molecular weight. This holds the polymer chains in position with a stronger force, and the increased interchain interaction and chain entanglement prevents densification.

The improved process stability and increased resistance to densification of the high molecular weight PBI is supported by the results of the drying study, shown in **Section 5.3.3**. In this study, the crosslinked standard molecular weight membrane suffered from increased permeance loss compared to the crosslinked high molecular weight membrane, despite having a larger pore size. The lower permeance loss is attributed to the increased interchain interactions and chain entanglement of the high molecular weight membranes which reduces the extent of pore collapse and densification. This shows that the polymer molecular weight is critically important to the process stability of membranes for OSN and OSU.

An alternative theory for the improved process stability of the high molecular weight membranes could be that the specific porosity or pore size of the membranes improves the process stability. However, the M0 membrane had a pore size intermediate between M16 and M18, and a permeance was between the permeance of M14 and M16. Therefore, if the lack of process stability was due to the high porosity, the M14 membrane should also show permeance decline, and if it was due to the small pore size, the M18 membrane should also show permeance decline. This was not observed. Therefore, the porosity or pore size cannot be the reason for the process instability.

A noteworthy result from this study is that the extent of crosslinking required to make the PBI membrane chemically stable in soak tests was significantly lower than the extent of crosslinking required for process stability. Thus, determining the chemical stability of a membrane through long-term soak tests does not accurately represent the true stability of a membrane for organic solvent separations. Instead, the true process stability can only be measured with long-term operation in the

process solvent. Having shown this for PBI membranes crosslinked with DBX, it is interesting future work to determine if this is true for other polymer types and crosslinkers.

5.3.5 Long-Term Stability at High Temperature

The long-term stability experiments showed that membranes manufactured from a high molecular weight PBI polymer had improved process stability and were process stable in DMF, with 24 h or longer crosslinking time. Although this was attributed to polymer chain rearrangement, the process instability of these membranes could instead be due to mechanical instability. Long-term storage of these membranes soaked in DMF shows the effect of chain mobility and ageing without the influence of pressure or mechanical instability. Performing this long-term storage at an elevated temperature increases the rate of polymer chain motion, and results in faster membrane ageing [253]. This was used to determine if the permeance decline was due to mechanical instability. For this work, 120 °C was chosen as the high temperature storage temperature because temperatures above this started to degrade the polypropylene backing material.

The first investigation was to confirm if the membranes were still chemically stable at 120 °C in DMF, shown in **Fig. 5.7a**. The chemical stability in DMF of the PBI membranes at high temperature was not significantly different to the chemical stability in DMF at 30 °C. Although, the uncrosslinked membranes fully dissolved, all crosslinked membranes were chemically stable. The membrane stability was also investigated by measuring the FT-IR signal of the membranes after storage at 120 °C in DMF, shown in **Fig. 5.7b**. By FT-IR, the low and high molecular weight membranes after 168 h of high temperature storage were identical to before the storage, showing that no chemical changes were occurring in the membrane.

To investigate the effect of mechanical instability on the membranes, the membranes were soaked in DMF at 120 °C for 168 h. The permeance of the membranes stored at elevated temperature was compared to the permeance of the membranes without heat treatment, shown in **Fig. 5.7c**. The high temperature storage resulted in the significant decline in the permeance of the standard molecular weight membranes. This permeance loss was highest for the 4 h crosslinked membrane and lower, but

still significant, in the 24 h and 48 h crosslinked membranes. Similarly, the high molecular weight membranes after 4 h crosslinking also showed some permeance decline. However, the permeance of the high molecular weight membranes crosslinked for 24 h and 48 h was unchanged after high temperature treatment. This matched closely to the trends in permeance decline under continuous pressurised filtration and showed that the process instability of the standard molecular weight membranes was due to polymer chain rearrangement and densification in the strong solvent, not due to mechanical instability, or the influence of continuous pressurisation.

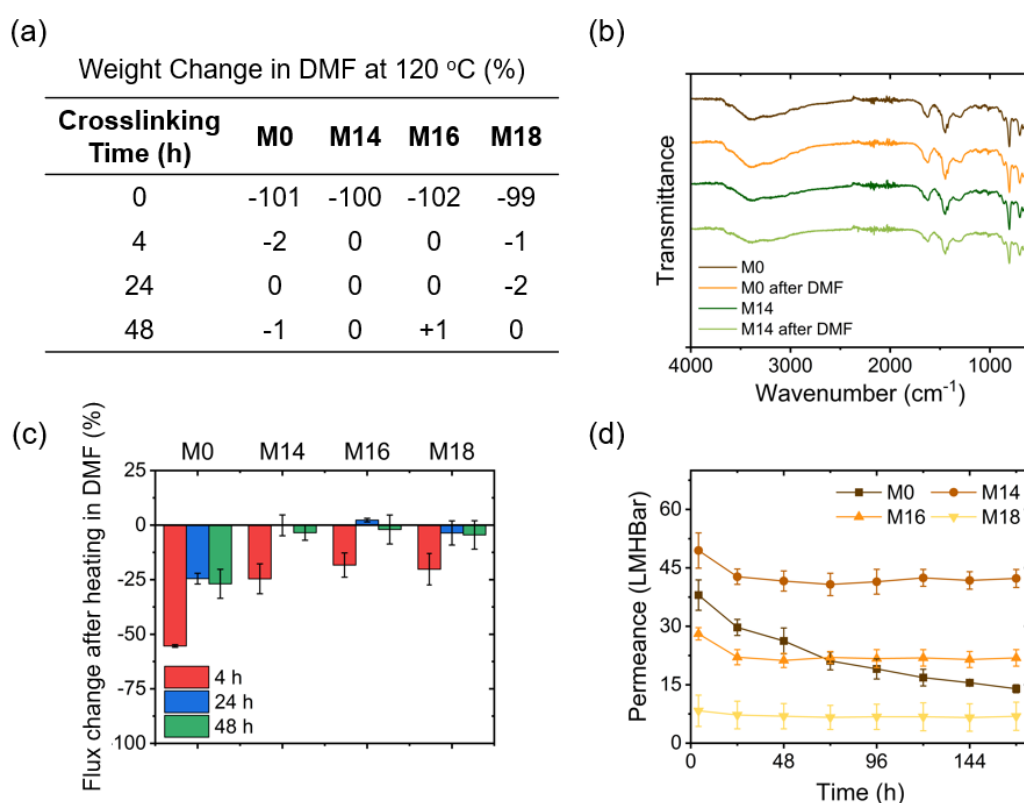


Fig. 5.7 (a) The chemical stability of the PBI membranes shown by the weight change after soaking in DMF at 120 °C for a minimum of four weeks. (b) The FT-IR of the 48 h crosslinked M0 and M14 membranes before and after storage in DMF at 120 °C for 168 h. (c) The change of the flux of the crosslinked PBI membranes after storing in DMF at 120 °C for 168 h, relative to the fresh membranes. The flux was measured in DMF at 60 L h⁻¹, 30 °C, 5 bar. (d) The permeance of the 48 h crosslinked PBI membranes in DMF at 120 °C. The permeance was continuously measured over 168 h pressurised filtration in stirred cells at 750 rpm and 5 bar.

If these PBI membranes were to be employed in an industrial process, the process stability over a longer time period needs to be verified. To determine the longer-term process stability of the membranes, the membranes were tested with continuous permeation at an elevated process temperature, of 120 °C. The elevated temperature was used to speed up the mobility of the PBI chains and was used to simulate the effect of long-term operation of the PBI membranes. The continuous performance of the 48 h crosslinked membranes at elevated temperature was investigated as these are the membranes most likely to be used industrially and need to have proven performance over an extended period. In previous studies, the standard molecular weight PBI has been shown to be unstable and have significant flux decline in DMF at elevated temperature, after crosslinking with DBX [265].

The permeance of the membranes was measured during 168 h continuous pressurised operation at 120 °C, shown in **Fig. 5.7d**. The standard molecular weight membrane showed constant permeance decline, which was attributed to the lack of process stability due to the fast rearrangement of the polymer chains towards a stable equilibrium position. Conversely, the high molecular weight PBI membranes all showed consistent permeance throughout the 168 h experiment, after 24 h compaction. This further demonstrates that using a high molecular weight polymer produces a membrane with significantly improved long-term process stability. This enables DBX crosslinked PBI to be used for high temperature or long-term separations in DMF, which was not possible with the standard molecular weight membrane.

After 168 h continuous pressurised filtration at 120 °C, the cross-section of the 48 h crosslinked membranes was investigated by SEM and compared to the cross-section of the untested membranes, shown in **Fig. 5.8**. The standard molecular weight PBI membrane showed significant deformation of the top layer after continuous filtration at high temperature, shown in **Fig. 5.8a**. Additionally, the finger-like structure showed signs of collapse and the entire structure appeared densified. The change of the cross-section structure of the standard molecular weight PBI confirmed that the polymer chains were susceptible to motion and the membrane was densified in DMF at an elevated temperature.

Conversely, the high molecular weight membranes appeared relatively unchanged after the high temperature filtration, shown in **Fig. 5.8b-d**. All high molecular weight membranes showed some densification of the top layer, possibly due to compaction or a small amount of initial ageing. However, the top layer was mostly unchanged, and the finger-like structure was mostly unaffected after long term continuous filtration at elevated temperature. This confirmed that using a high molecular weight polymer reduces chain mobility and densification, which resulted in improved process stability compared to the standard molecular weight membranes. This shows that the polymer molecular weight is critical to the long-term performance of DBX crosslinked PBI membranes.

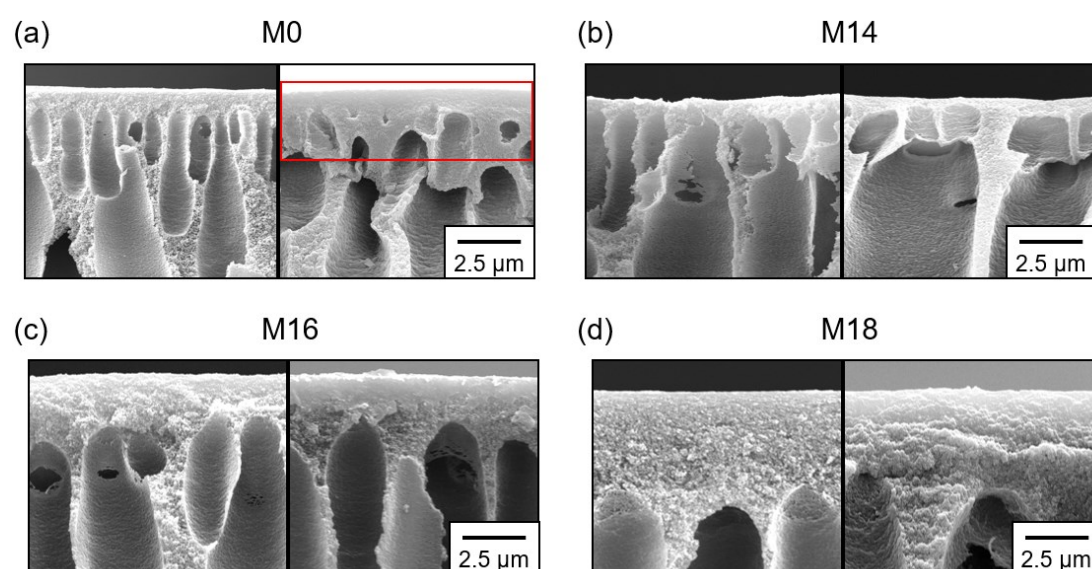


Fig. 5.8 SEM of the top surface of 48 h crosslinked PBI membranes before (left) and after (right) 168 h continuous pressurised DMF filtration at 750 rpm, 5 bar, 120 °C of (a) M0, (b) M14, (c) M16, (d) M18. The red box shows the significantly densified selective layer of M0.

5.4 Conclusions

Two different molecular weights of PBI were used to manufacture membranes for organic solvent separations. The standard molecular weight PBI, 27000 g mol⁻¹, which is commonly used for organic solvent separations, was used, along with a high molecular weight PBI, 60000 g mol⁻¹, which is untested for organic solvent separations. Membranes cast from the high molecular weight PBI had a significantly lower permeance than the standard molecular weight PBI for the same MWCO. This was due to the

higher viscosity of the dope solution which led to a slower phase inversion. However, by changing the concentration of the dope solution, the permeance and MWCO of the high molecular weight membrane were tuneable.

The membranes from the two polymer molecular weights were crosslinked with DBX and the process stability of these membranes was tested. Both molecular weights of crosslinked PBI membrane were chemically stable in DMF, determined through long-term soak tests. Additionally, both molecular weights of crosslinked PBI membrane were shown to be mechanically stable. However, only the high molecular weight PBI membranes were process stable in DMF. Over 168 h continuous filtration, the high molecular weight membrane had constant permeance, while the permeance of the standard molecular weight membrane slowly decreased. The increased process stability of the high molecular weight membrane was attributed to the increased chain entanglement and interchain interactions of the high molecular weight polymer, which prevented densification and ageing. This was further demonstrated by increasing the operating temperature of the PBI membranes to 120 °C. The high molecular weight membranes were process stable with no permeance decline at 120 °C. However, the standard molecular weight membrane suffered 50% permeance loss due to the enhanced rate of chain rearrangement and densification at an elevated temperature.

This work showed that the polymer molecular weight is critical to the stability of PBI membranes in strong organic solvents. Additionally, this work shows that there is a minimum process stability which is required to reduce membrane ageing, and this is significantly higher than the chemical stability determined by soak tests.

Chapter 6: Anti-fouling membranes for organic solvent nanofiltration and organic solvent ultrafiltration: graft modified polybenzimidazole

Abstract

Fouling is one of the major obstacles to successful membrane-based separations. In aqueous systems, fouling is well characterised and fouling reduction techniques are established. However, in organic solvents, membrane fouling is not well understood. In this work anti-fouling organic solvent stable polybenzimidazole (PBI) membranes were manufactured by graft modifying the surface with linear poly(ethylene glycol) (PEG) and poly(propylene glycol) (PPG). Graft modification provided an effective method of reducing fouling. However, the anti-fouling properties were dependent on the interactions between the modifying polymer and the solvent. In water, the hydrophilic PEG graft modifiers had favourable modifier-water interactions and provided an anti-fouling steric layer on the surface of the membrane. However, hydrophobic PPG graft modifiers had unfavourable modifier-water interactions and provided no anti-fouling properties. Conversely, in polar organic solvents, graft modification with PPG showed superior modifier-solvent interactions, and superior anti-fouling properties, relative to graft modification with the same molecular weight PEG. In non-polar solvents, both the PEG and PPG modifiers had unfavourable modifier-solvent interactions and provided minimal anti-fouling properties. The anti-fouling properties after graft modification were closely correlated to the hydrodynamic diameter of the grafting polymer in solution, and the selection of an optimal grafting polymer for manufacturing anti-fouling OSN and OSU membranes is significantly solvent dependant.

Keywords: Fouling, Graft Modification, Organic Solvent Nanofiltration (OSN), Organic Solvent Ultrafiltration (OSU), Polybenzimidazole (PBI)

Highlights

- Crosslinked PBI membranes were stable in a range of polar and non-polar solvents
- These membranes were chemically graft modified with linear PEG and PPG
- Graft modification provided a method of reducing membrane fouling
- The anti-fouling properties were significantly dependant on the process solvent
- Greater extension of the modifier into solution gave better anti-fouling membranes

6.1 Introduction

Fouling is a problem which is inevitable in almost all membrane-based separation processes [284,285]. Fouling is the accumulation of particles or molecules on the top surface, or inside the pores of a membrane. The deposition of these particles or molecules leads to the decline of the permeance, and often the selectivity, of a membrane over time [286,287]. This has a significant impact on the economics of membrane separations as it leads to an increased operating cost for energy, maintenance, cleaning chemicals, labour, and inevitably shorter membrane lifetimes [288,289].

Due to the uptake of membranes for water purification, the rate and extent of fouling has been studied in aqueous systems. Fouling is known to be dependent on many properties, including feed composition (foulant type, concentration, pH), system properties (flux, temperature, spacer design) and membrane properties (roughness, hydrophilicity, surface charge) [290–297]. Techniques to predict, quantify, and reduce fouling have been extensively studied for aqueous systems and ‘anti-fouling’ membranes have become relatively commonplace. These anti-fouling aqueous membranes use many techniques to reduce fouling, including changing the surface roughness, charge, and hydrophilicity of the membrane [210,298–305].

In contrast, membrane separations in organic solvents are a relatively new field of interest, with applications in the petrochemical, pharmaceutical and fine chemical industries [8,11,31,253]. In organic solvent nanofiltration (OSN) and organic solvent ultrafiltration (OSU), the use of different solvents and applications will have a significant impact on the most common types of foulant, the rate of fouling, and the viability of many anti-fouling techniques [9,306–308].

There have been some reports of specific examples of fouling in organic solvents. Sigurdardóttir et al. found that the permeance decline due to fouling in alcohol solutions was a function of the pH, with higher pH leading to a higher tendency for fouling [309]. Shi et al. investigated fouling of highly concentrated oil solutions [310]. They found that a viscous oil layer formed on the surface of polyimide membranes and plugged the membrane pores. This could be somewhat mitigated by using cross-flow cells with high flow rates, which achieved permeances five times higher than dead end cells. Zhao et

al. investigated backflushing of hollow fibre OSN membranes, restoring the majority of the methanol permeance after fouling [311].

Due to the wide range of potential solvents, foulants, and membranes used in OSN and OSU, general fouling trends are extremely important, and have started to be investigated. Yin et al. used colloidal silica and polyacrylonitrile (PAN) membranes to measure fouling [312]. They found that the zeta potential was a poor indicator of fouling. They also found that higher polarity solvents had more repulsive interfacial interactions between the membrane and colloidal silica, which led to reduced fouling, while non-polar solvents had attractive interfacial interactions, which led to higher fouling. This work was furthered by comparing Derjaguin-Landau-Verwey-Overbeek (DLVO) and extended-DLVO (XDLVO) theories, as well as zeta potential to experimental fouling results, with DLVO showing the closest fit to the experimental flux decline [313,314]. Molecular dynamic simulations have also been used to quantify the effect of fouling in different solvents, showing the significant influence of the solvent type on membrane fouling [315].

In all cases, fouling is inevitable and anti-fouling, or foulant resistant, OSN and OSU membranes will significantly improve the cost efficiency, energy consumption, and lifetime of a membrane. One anti-fouling method which is well established in aqueous systems is the grafting of polymer chains to the surface of the membrane [201,223,316–319]. This provides a steric barrier which prevents the adsorption of colloids and proteins. Often poly(ethylene glycol) (PEG) is used for grafting as it is hydrophilic and it forms a large hydration layer to give additional steric resistance [207,228,232,320,321]. However, this technique is used almost exclusively in aqueous systems. In organic solvent systems, polymer graft modification has been shown to be a good technique for tuning the selectivity and permeance of a membrane [139,224,263,322]. However, the anti-fouling characteristics of polymer grafted membranes in different solvents have not yet been studied.

It was hypothesised that anti-fouling membranes for organic solvent separations can be manufactured by grafting polymer chains to the top surface of polybenzimidazole (PBI) membranes, as shown in **Fig. 6.1**. PBI was chosen due to its high mechanical and chemical stability after crosslinking

[12,189,199,263]. Different ratios of poly(ethylene glycol) (PEG) and poly(propylene glycol) (PPG) were used to vary the hydrophilicity of the grafted polymers, along with varying the PEG/PPG molecular weight. These membranes were tested in a range of solvents, with a colloidal silica foulant, to investigate the effect of different polymer graft compositions on the anti-fouling properties of PBI membranes. These anti-fouling membranes should have a longer lifetime and more consistent performance in DSP synthesis, therefore, improving the reliability and reducing the cost of the process.

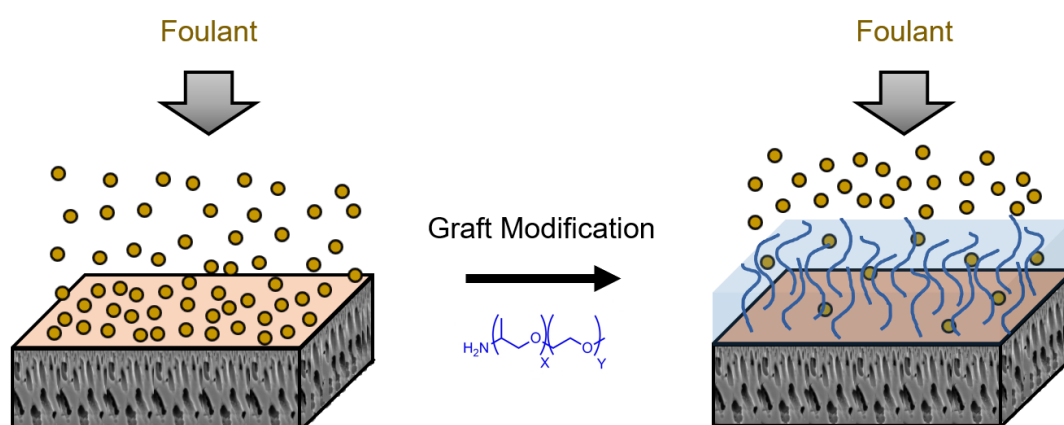


Fig. 6.1 A schematic showing the proposed polymer layer after graft modification with linear polyether monoamine, which is intended to provide steric resistance to foulant in organic solvents.

6.2 Materials and Methods

6.2.1 Materials

A custom solution of PBI (1.1 IV powder dissolved at 16 wt% with 1.5 wt% lithium chloride in N,N-dimethylacetamide) was purchased from PBI Performance Products (USA). Novatexx 2471 non-woven support, made of polypropylene, was purchased from Freudenberg Filtration Technologies (UK). HPLC grade acetonitrile (MeCN), propan-2-ol (IPA), toluene, methanol (MeOH), and water for membrane synthesis and membrane testing were purchased from VWR (UK). N,N-dimethylacetamide, α,α' -dibromo-*p*-xylene (DBX), N,N-diisopropylethylamine and PEG (400 g mol⁻¹) for membrane synthesis were purchased from Sigma Aldrich (UK). PEG (400, 1000, 2000, 4000, 8000 and 35000 g mol⁻¹) for membrane testing was purchased from Sigma Aldrich (UK). Graft modifiers RE1-1000 (1kPEG), RE1-2007 (2kPEG), and M-2005 (2kPPG), were kindly donated by Huntsman (UK). 20 nm silica

nanoparticles were purchased from General Engineering and Research (USA) as a 10 wt% aqueous dispersion.

6.2.2 Methods

6.2.2.1 Casting

Unless specified, the membranes were cast with 16 wt% PBI solution, used as received. For the membranes in **Section 6.3.5**, a 12 wt% solution was used. For the 12 wt% solution, the 16 wt% PBI solution was diluted to 12 wt% with N,N-dimethylacetamide. Each PBI solution was cast using the technique described in **Section 4.2.2.1** at a minimum length of 6 m.

6.2.2.2 Crosslinking and Modification

The crosslinking reactions were performed with the method described in **Section 4.2.2.2**, with a 0.15 m × 0.4 m membrane roll in a 160 ml cylinder, maintained at 80 °C for 24 h.

After crosslinking, the PBI was immersed in acetonitrile containing a hindered base, N,N-diisopropylethylamine (0.6 mol L⁻¹). The graft modifier: 1kPEG, 2kPEG, or 2kPPG (0.2 mol L⁻¹) was added and the solution was heated at 80 °C under an argon atmosphere. After 24 hours the solution was cooled to room temperature and the membrane washed three times with fresh acetonitrile. After modification, the membranes were preserved with PEG 400 using the method described in **Section 4.2.2.4**.

6.2.2.3 Characterisation

For contact angle, specific weight change, and SEM, the methods described in **Section 4.2.2.6** were used, except with the vacuum oven at 100 mbar. For FT-IR and liquid-liquid displacement porometry, the methods described in **Section 5.2.2.3** were used.

For XPS elemental analysis, the membranes were washed three times with acetonitrile, then dried in a vacuum oven overnight at 80 °C and 100 mbar (Fistreem). Samples were measured with a Thermo Scientific K-alpha XPS equipped with a microfocussed monochromated Al X-ray source. The source

was operated at 12 keV and a 400 μm spot size was used. The analyser was operated at a constant analyser energy of 200 eV.

The size of the modifying polymers was measured using an Anton Parr Litesizer 500 with a glass cuvette. 5 wt% of each polymer was dissolved in each solvent and a minimum of five measurements were taken for each sample.

6.2.2.4 Performance Testing

The performance testing was performed on the rig described in **Section 4.2.2.8**. Four membranes of each type were tested. The rejection was measured using 1 g L⁻¹ each of PEG 400, 1000, 2000, 4000, 8000, and 35000 g mol⁻¹. The rig was operated at a cross-flow rate of 60 L h⁻¹ per parallel path, and membrane compaction was completed at 20 bar for a minimum of 24 hours prior to sampling. This time was found to be sufficient to produce constant permeance and rejection after initial physical aging of the membrane.

6.2.2.5 Fouling Testing

To solvent exchange the colloidal silica to a second solvent, a minimum of 10 mL aqueous nanoparticles was diluted in the second solvent in a 200 mL diafiltration rig. Constant volume diafiltration was completed by continuous addition of a second solvent and permeation through two 14 cm² discs of 2kPEG membrane. The rig was operated at a cross-flow of 48 L h⁻¹ using a Knauer Azura P 2.1L HPLC pump and a minimum of eight system volumes were permeated at 5 bar. After permeation, the rig was thoroughly washed, and the concentration of the silica nanoparticles in the second solvent was calculated by mass balance. For solvent exchange to toluene, a two-step method of solvent exchanging to methanol and then to toluene was completed using the procedure above.

Fouling experiments were conducted in the same rig as the rejection testing, except using only one path made up of four cells, each with an area of 14 cm². The rig was operated at 60 L h⁻¹ cross-flow rate and 30 °C. Four membranes of each type were compacted for a minimum of 24 hours at 20 bar. After compaction, 50 ppm 20 nm silica nanoparticles were added to the rig and permeated at an initial flux of 200 L m⁻² h⁻¹ for eight hours.

Additional experiments with varying conditions were conducted in the same rig with a minimum of four cells. The flow rate was varied to as low as 40 L h⁻¹ and with as much as 500 ppm silica nanoparticles in order to determine the effect of changing conditions on the rate of fouling. All other conditions, except the changed parameter, were kept constant.

6.3 Results and Discussion

6.3.1 Membrane Modification

The proposed crosslinking and modification of PBI membranes is shown in **Fig. 6.2a**. The first step is an electrophilic alkylation of the PBI with DBX. This partially crosslinks the PBI membrane and generates a bromomethyl reactive site within the membrane matrix (**Fig. 6.2a**, Reaction 1). Some of these bromomethyl reactive sites react with a second benzimidazole monomer to crosslink the PBI membrane (**Fig. 6.2a**, Reaction 2). However, depending on the local concentration of DBX and the crosslinking time, there will be a distribution of partially reacted and completely crosslinked DBX remaining in the membrane at the end of the crosslinking reaction. The partially crosslinked DBX provides residual reactive bromomethyl sites within the membrane matrix which can be used for graft modification.

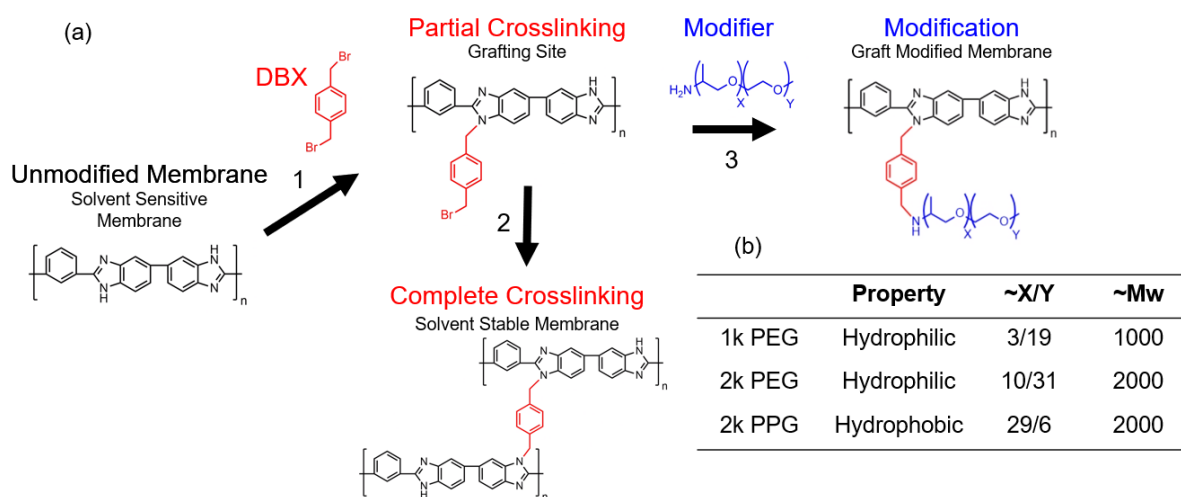


Fig. 6.2 (a) The proposed crosslinking and modification reactions to manufacture solvent stable graft modified PBI membranes. (b) The properties of the graft modifiers used for modification of the PBI membranes.

The second step in the manufacture of the anti-fouling membranes is graft modification of a polyether monoamine onto the residual reactive bromomethyl sites (**Fig. 6.2a**, Reaction 3). In this work three different modifiers were used for grafting, shown in **Fig. 6.2b**. 1kPEG and 2kPEG are both hydrophilic PEG-based polymers of 1,000 and 2,000 g mol⁻¹ respectively. 2kPPG is a hydrophobic PPG-based polymer of 2,000 g mol⁻¹.

The successful modification of the PBI membranes is characterised in **Fig. 6.3**. **Fig. 6.3a** shows the specific weight change in the modification reactions. Modifying with 1kPEG resulted in a 22% increase in the PBI weight, from 33.9 g m⁻² to 41.4 g m⁻², due to the integration of the graft modifier into the membrane matrix. This increased to a 36% and 39% weight change after modification with 2kPEG and 2kPPG respectively, proving that the modification was successful. The weight increase for the 2,000 g mol⁻¹ modifiers was less than a two-fold increase compared to the 1,000 g mol⁻¹ modifier. This showed that there was a lower extent of modification for the 2,000 g mol⁻¹ modifiers than the 1,000 g mol⁻¹ modifiers. It is hypothesised that the higher molecular weight modifiers have additional steric bulk which slows their diffusion into the membrane matrix and decreases their reactivity, therefore resulting in a lower total extent of modification than the 1,000 g mol⁻¹ modification. The total extent of grafting was calculated from the weight change of the membranes during the modification. This was calculated to be 0.25, 0.21, and 0.22 mmol of modifier per gram of PBI polymer for 1kPEG, 2kPEG, and 2kPPG respectively.

The successful modification was corroborated by investigating the elemental composition of the membranes, shown in **Fig. 6.3b**. After modification, the bromine content of the membrane decreased for all three modifiers. This was due to the reaction of the bromomethyl sites with the graft modifier. Successful grafting of the PBI membranes displaces the bromine in the membrane matrix and leads to a reduction in the bromine content after modification. Additionally, for all modifiers the nitrogen and carbon content were decreased during the modification, due to the higher carbon and nitrogen content of the crosslinked PBI relative to the PEG or PPG modifier. There was also a significant increase in the

oxygen content of the membrane during modification. This is due to the high oxygen content of PEG or PPG and demonstrates that the modification of the PBI membrane was successful.

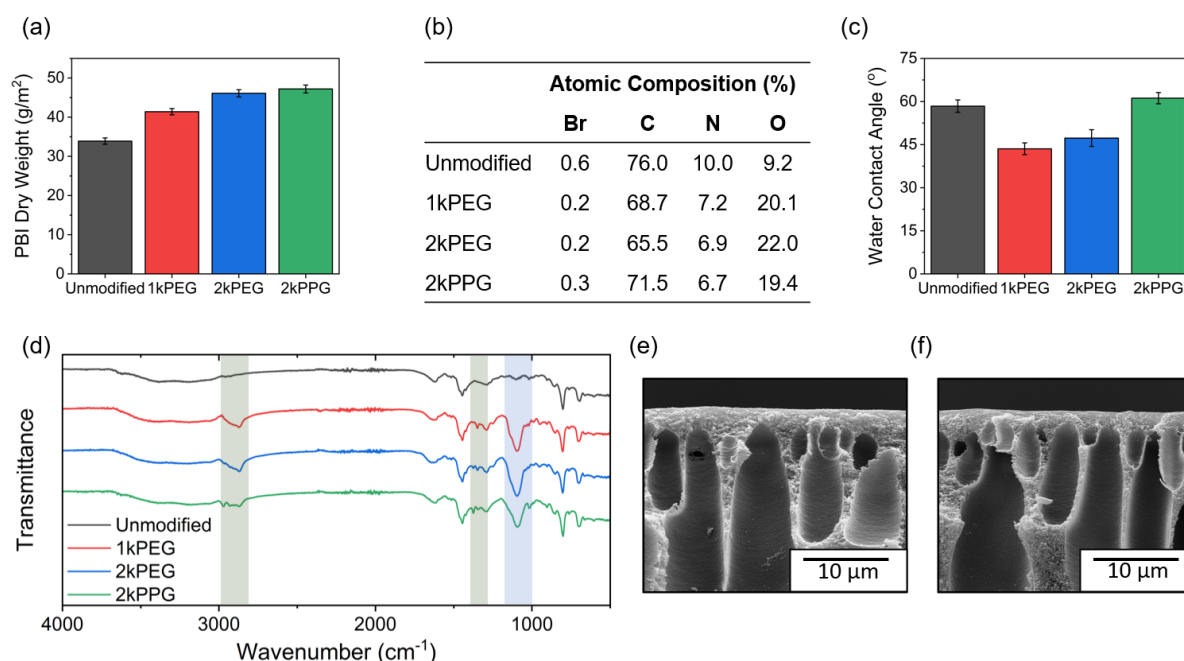


Fig. 6.3 (a) The specific weight of the dry PBI membranes. (b) The atomic elemental analysis (%) of the membrane, measured using XPS (c) The water contact angle of the PBI membranes. (d) FT-IR of the crosslinked and modified membranes. (e) SEM of the cross section of the unmodified PBI membrane. (f) SEM of the cross section of the 2kPPG modified PBI membrane.

Additionally, grafting was investigated by measuring the change in the water contact angle, shown in **Fig. 6.3c**. A slight increase in the contact angle to 61 ° was observed with 2kPPG modification, compared to 58 ° for the unmodified membrane. This was due to the relatively hydrophobic nature of the PPG-based modifier. As the hydrophilicity of the modifier was increased, the water contact angle decreased to 47 ° for 2kPEG and 43 ° for 1kPEG. The higher contact angle of the 2kPEG modified PBI was due to the higher PPG:PEG ratio than 1kPEG.

The grafting to the PBI membranes was also characterised with FT-IR, shown in **Fig. 6.3d**. The unmodified membrane showed a broad peak between 2500 cm⁻¹ and 3600 cm⁻¹, and a sharp peak at 1630 cm⁻¹. These peaks are characteristic of PBI and correspond to heterocyclic imidazole [196,272]. After modification, the membranes had a significant new FT-IR peak at 1104 cm⁻¹, which was present

for all modifiers. This is characteristic of an ether bond and shows the presence of PEG or PPG in the membrane matrix. Additionally, after PEG modification there were two peaks at 2870 cm^{-1} and 1345 cm^{-1} . These corresponded to C-H and were not shown in the unmodified membrane. Conversely, after PPG modification, an additional peak was identified around 2950 cm^{-1} and the C-H peak was shifted from 1345 cm^{-1} to 1375 cm^{-1} . These peaks are characteristic of PEG and PPG respectively and showed that modification was successful, with a clear spectral difference between modification with PEG and PPG [323].

The SEM cross section of the PBI membranes before modification, shown in **Fig. 6.3e**, and after modification with 2kPPG, shown in **Fig. 6.3f**, were compared. These SEM images showed that there was no significant change to the membrane selective layer or the underlying finger-like structure in the modification reaction. Therefore, all changes to the membrane properties and performance are due to the grafting of PEG or PPG, not due to changes in the membrane morphology.

6.3.2 Foulant Characterisation

20 nm colloidal silica nanoparticles were used as a model foulant. Silica was chosen in line with previous studies on fouling in organic solvent systems [312]. The size of the silica nanoparticles was selected due to the similar size of the foulant observed during oligonucleotide synthesis in **Chapter 7**. To ensure the silica was dispersed effectively, the silica particle size was measured in each solvent used in this work. **Fig. 6.4a** shows the particle size distribution in water, methanol, acetonitrile, and toluene, calculated using SEM. An example SEM image of the silica nanoparticles in acetonitrile is shown in **Fig. 6.4b**. The average particle size was approximately 25 nm in all tested solvents and the particle size distribution showed that the silica particles were consistent in all tested solvents.

The pore size of the unmodified membrane was approximately 3.9 nm, measured using liquid-liquid displacement porometry. Therefore, it was assumed that the silica nanoparticles had 100% rejection. This was confirmed by evaporating 100 mL permeate per membrane and weighing the residual mass. All membranes showed over 99% rejection for the colloidal silica nanoparticles in all solvents.

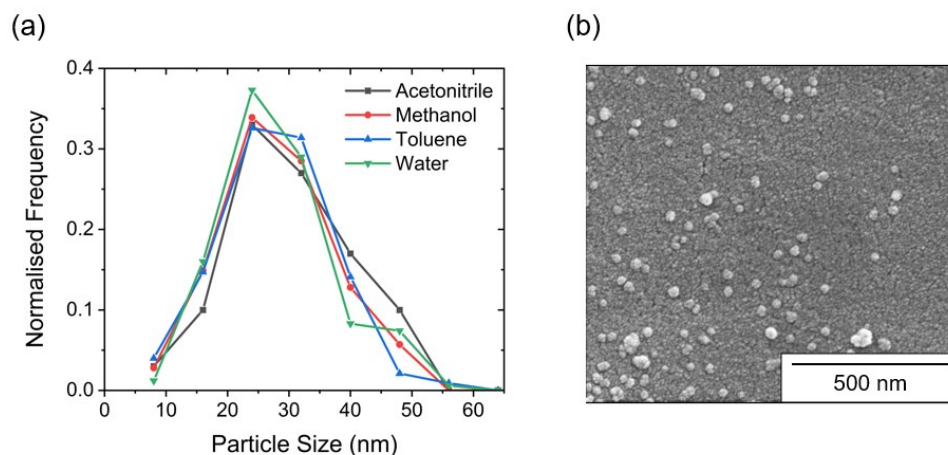


Fig. 6.4 (a) The particle size distribution of the silica particles in each solvent, measured using SEM image analysis. (b) An example SEM image of the silica nanoparticles recovered from acetonitrile.

6.3.3 Membrane Fouling

Fig. 6.5 shows the flux decline of each of the membranes in four tested solvents: water, acetonitrile, methanol, and toluene. These solvents were chosen to represent a wide range of polarities, protic and aprotic solvents, as recommended in previous studies [8]. To ensure the flux decline was due to fouling, and not compaction or ageing, the permeance was measured 20 hours prior to the addition of the silica nanoparticles. This was compared to the permeance just before the silica particles were added. For all membranes, in all solvents, there was less than 3% change in permeance over the 20 hour period. From this, it was assumed that all changes to the permeance were due to fouling and not membrane ageing.

Fig. 6.5a shows the flux decline in water. The unmodified membrane showed a significant flux decline in water. After eight hours permeation with the colloidal silica foulant the flux was decreased to 75% of the initial pure water flux. Comparatively, the 1kPEG modified membrane showed a significant anti-fouling effect, with the flux only decreased to 93% of the initial flux for the same conditions. Increasing the length of the modifier to 2kPEG resulted in a small improvement of the anti-fouling properties, with the flux decreased to 95% of the initial pure water flux. This showed that PEG graft modification provided a significant anti-fouling benefit, which agrees with many literature sources on anti-fouling membranes in aqueous systems. These anti-fouling properties are often attributed to the creation of a

physical barrier which exhibits a significant steric repulsive force. Compression of the PEG in this barrier is unfavourable and results in a repulsive elastic force which prevent fouling. Additionally, the PEG barrier has a hydrogen bonded hydration layer and fouling is energetically unfavourable because it requires removal of this layer [200,210,324].

Conversely, modification with a more hydrophobic modifier, 2kPPG, provided almost no anti-fouling properties in water. The flux of the 2kPPG modified membrane decreased to 77% of the initial pure water flux, which was similar to the flux decline of the unmodified membrane. This result was vastly different to graft modification with a similar length PEG, and the increased fouling is attributed to the different modifier-water interactions. PEG has highly favourable modifier-solvent interactions and the PEG-based modifier extends away from the membrane surface. This provides a continuously moving steric barrier and hydration layer which prevents fouling of the membrane [324]. Conversely, PPG has unfavourable modifier-water interactions. This makes PPG insoluble in water and the modifier does not extend away from the membrane surface. Instead, the PPG collapses into a tight conformation onto the surface of the PBI membrane [236,237]. Therefore, the PPG provides no steric barrier layer to foulant particles, which results in a high degree of fouling, similar to the unmodified membrane [325]. Hence, in aqueous systems PEG-based modification, not PPG based-modification, is the superior graft modifier for anti-fouling membranes.

The flux decline due to fouling was also measured in two different polar organic solvents, namely acetonitrile and methanol, shown in **Fig. 6.5b** and c. Similar to in water, in polar solvents, PEG graft modification resulted in a significant anti-fouling benefit. In acetonitrile, the relative flux of the unmodified membrane decreased to 66% of the pure solvent flux, while the 1kPEG and 2kPEG decreased significantly less, to 77% and 86% respectively. In methanol the flux decreased to 81%, 84%, and 87% of the pure solvent flux for the unmodified, 1kPEG and 2kPEG membranes respectively. This was a similar result to the anti-fouling effect in water and showed that increasing the length of the modifier increased the size of the continuously moving steric barrier and improved the anti-fouling effect of the membrane [326]. This was verified by measuring the hydrodynamic diameter of the graft

modifiers in each solvent, shown in **Table 6.1**. In water, acetonitrile, and methanol the hydrodynamic diameter of 2kPEG was higher than the hydrodynamic diameter of 1kPEG. For all membranes, the larger size of 2kPEG in solution correlated to an improvement of the anti-fouling properties of the membrane. This was attributed to the 2kPEG creating a larger steric barrier which also provides an increased repulsive force to the foulant when compressed [327].

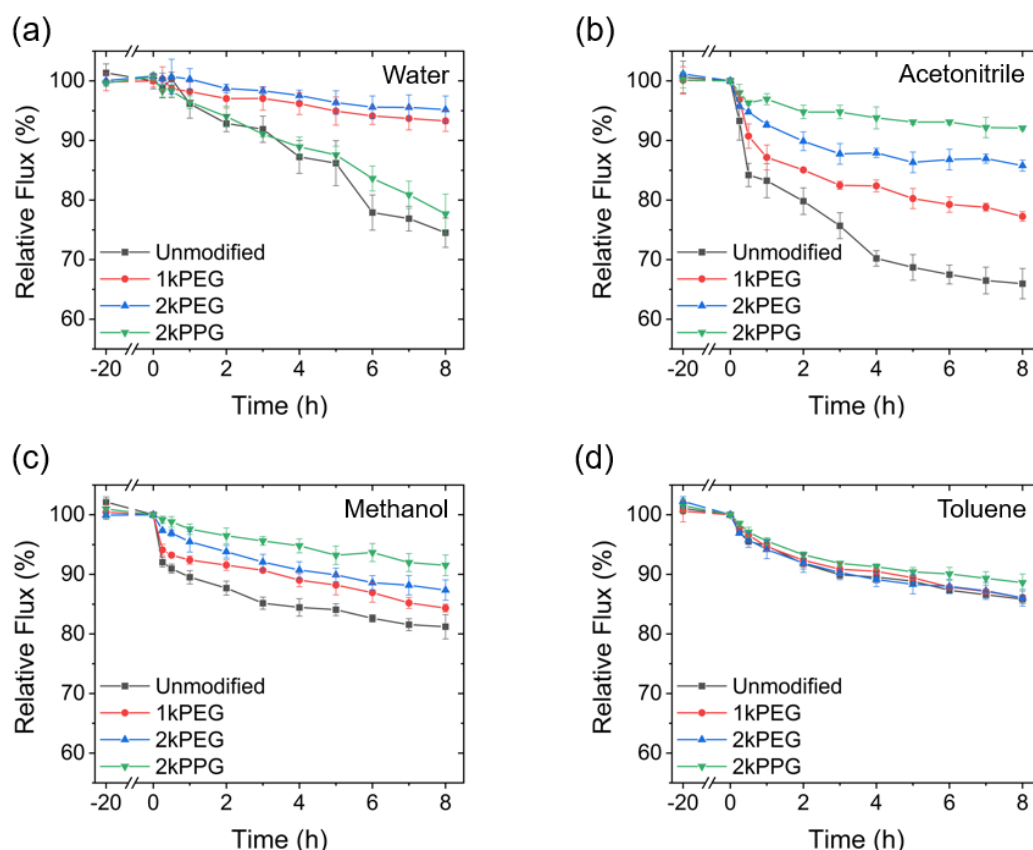


Fig. 6.5 The relative flux of the unmodified and modified membranes in (a) water, (b) acetonitrile, (c) methanol, and (d) toluene. The initial permeance was set to approximately $200 \text{ L m}^{-2} \text{ h}^{-1}$ for each membrane and 50 ppm 20 nm silica nanoparticles were added at 0 hours. The system was operated at 60 L h^{-1} cross-flow rate and 30°C .

It is noteworthy that the anti-fouling properties of the 1kPEG grafted membranes were better in water > methanol > acetonitrile. This was attributed to the strongly bound PEG hydration in water, due to hydrogen bonding. This hydration layer creates a large energy barrier which resists fouling. This results in a low permeance decline in water [328,329]. Comparatively, there is no hydrogen bonding between

the PEG and the solvent in acetonitrile. This means that there is no strongly bonded solvent layer, and the anti-fouling properties are exclusively due to the PEG steric barrier. This barrier still provided a significant anti-fouling effect, and this shows that the creation of a steric barrier is an effective method of producing anti-fouling membranes in polar organic solvents which cannot hydrogen bond. However, the e hydrogen bonded hydration layer does result in improved anti-fouling properties in water and methanol compared to acetonitrile.

Table 6.1 *Hydrodynamic diameter of the polymers used for graft modification in each solvent.*

	Hydrodynamic Diameter (nm)			
	Water	Methanol	Acetonitrile	Toluene
1kPEG	2.05	2.16	1.79	<1.00
2kPEG	3.09	2.31	2.63	1.14
2kPPG	Insoluble	2.71	2.99	1.22

Using the 2kPPG modified membrane in polar organic solvents resulted in the flux of the fouled membrane decreasing to 92% of the pure solvent flux in both acetonitrile and methanol. This was a significant improvement compared to the anti-fouling effect of 2kPPG modification in water, which showed no anti-fouling properties due to the collapse of the insoluble 2kPPG modifier. Additionally, comparing the 2kPPG and 2kPEG grafted membranes in polar solvents, the PPG modified membrane showed superior anti-fouling properties for the same molecular weight of modifier. This is because PPG has more preferential modifier-polar solvent interactions. PPG is more hydrophobic, which results in PPG having a higher hydrodynamic diameter than the same molecular weight PEG polymer in polar solvents, as shown in **Table 6.1**. Therefore, in polar organic solvents, the PPG modifier has a more extended conformation. This results in the polymer extending further from the membrane surface which increases the size of the steric barrier, and compression of this barrier is energetically more unfavourable. This prevents foulant particles reaching the membrane surface, and consequently, PPG

modifiers are a much more effective method of manufacturing anti-fouling membranes than PEG modifiers in polar organic solvents.

To investigate the effect of graft modification in non-polar solvents, the flux decline was measured in toluene, **Fig 6.5d**. The relative flux for the unmodified membrane after fouling was 85% of the pure solvent flux. However, modifying the membranes with PEG did not significantly change the anti-fouling effect. The relative flux for both PEG modified membranes decreased to 86% of the pure solvent flux, which was similar to the unmodified PBI. Modifying with the more hydrophobic PPG showed a small increase in the anti-fouling properties, with the relative flux of the 2kPPG modified membranes decreasing less, to 89% of the pure solvent flux. However, this was not a significant change and showed that graft modification with PEG or PPG is not an effective method of creating anti-fouling membranes in toluene.

The PEG and PPG modifiers had a significantly smaller hydrodynamic diameter in toluene compared to water and the polar solvents, shown in **Table 6.1**. The hydrodynamic diameter of 1kPEG, 2kPEG, and 2kPPG were <1.0 nm, 1.14 nm, and 1.22 nm respectively. Therefore, the graft modifiers have more favourable affinity with themselves than with the solvent. This results in a significantly smaller steric barrier in toluene, which makes the PBI membranes more susceptible to fouling. Additionally, when the modifier is grafted to a PBI membrane, which is a relatively hydrophilic surface, the affinity of the modifier for the PBI could be greater than the affinity for the solvent. This causes the PEG or PPG graft modifier to collapse onto the surface of the membrane and results in a very small anti-fouling effect. It is believed that a more hydrophobic graft modifier than PPG is needed to show anti-fouling properties in non-polar solvents. The collapse of polar graft modifiers in non-polar solvents was previously reported in literature. However previous studies only investigated the effect on rejection, not the effect on fouling [263].

To investigate whether the flux decline was due to differences in the fouling of the PBI membranes, SEM images of the membranes fouled in acetonitrile were analysed, shown in **Fig. 6.6**. **Fig. 6.6a** shows that the unmodified membrane had the highest degree of fouling, and that most of the membrane surface

was covered with a significant quantity of foulant. The extent of fouling observed in the SEM images decreased sequentially with the modifications of 1kPEG, 2kPEG, and 2kPPG, shown in **Fig. 6.6b-d**. This matched the trend observed from the relative flux and showed that measuring the flux decline was an effective method of measuring the anti-fouling effect of the membranes. This also confirmed that graft modification decreased the rate of membrane fouling, and that the 2kPPG was the optimal modifier for improving the long-term performance of the PBI membranes in acetonitrile.

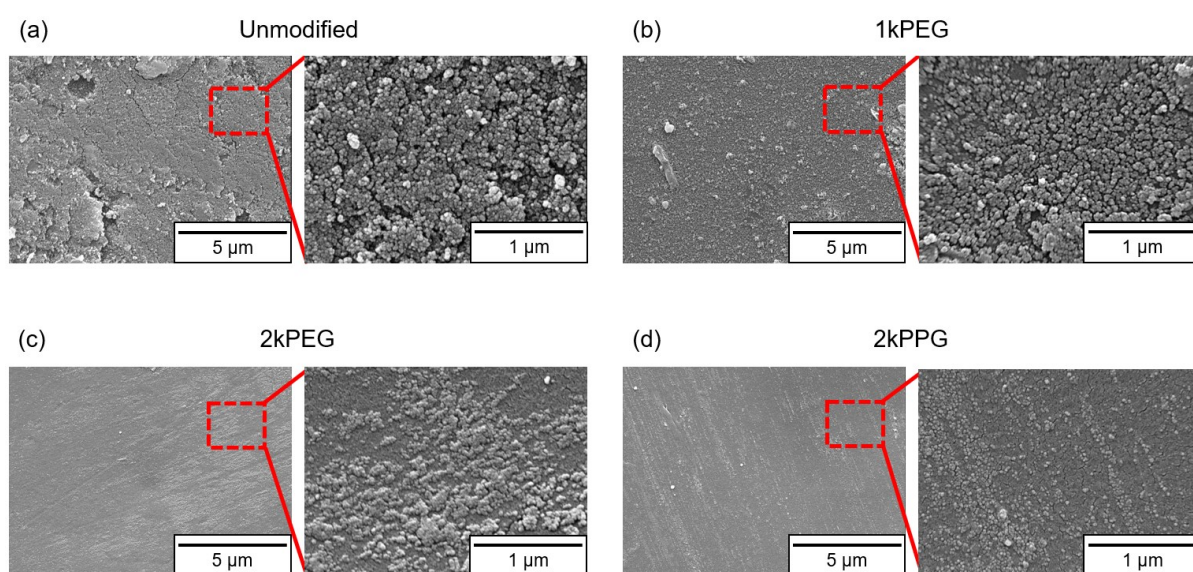


Fig. 6.6 SEM images of the top surface of the PBI membranes after eight hours fouling with 50 ppm silica nanoparticles in acetonitrile of the (a) unmodified membrane, (b) 1kPEG, (c) 2kPEG, and (d) 2kPPG.

The solvent dependency of fouling in OSN and OSU was also investigated in this study. **Fig. 6.7** compares the SEM images of the unmodified PBI membranes after silica fouling in different solvents. This study allowed assessment of the membrane's susceptibility to fouling in different solvents, without any influence of graft modification. It was observed that fouling in aqueous systems resulted in a relatively flat surface of silica particles with consistent fouling across the whole membrane surface. However, as the solvent polarity decreased, the fouled surface became rougher. In toluene, the fouled surface had the greatest roughness, and this is believed to be because the silica particles have preferential attractive interactions to other silica particles in lower polarity solvents, rather than to the surface of the PBI membrane. This foulant morphology is believed to be the reason for the relatively low flux decline

of the unmodified membranes in toluene, compared to water, methanol, and acetonitrile, shown in **Fig. 6.5**. The solvent dependency of fouling was previously observed for silica fouling on PAN [312] and shows that the three-way foulant-membrane-solvent interactions are critically important for studying membrane fouling in organic solvents.

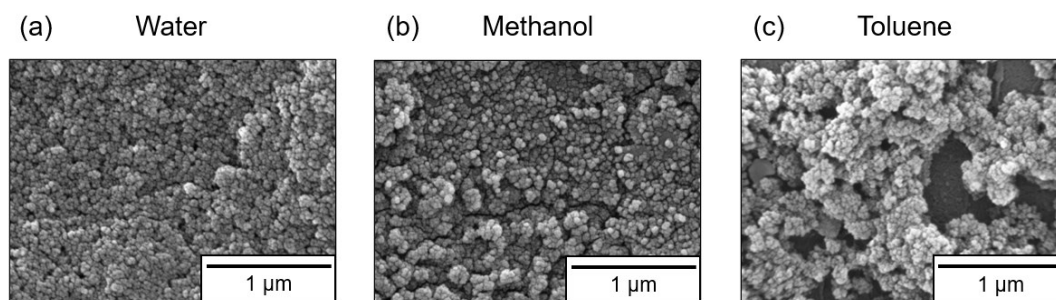


Fig. 6.7 SEM images of the unmodified PBI membranes after eight hours fouling with 50 ppm silica nanoparticles in (a) water, (b) methanol, and (c) toluene.

6.3.4 Membrane Performance

The rejection and permeance of the unmodified and modified membranes were tested, shown in **Fig. 6.8**. A standard marker series for OSN was used: a series of increasing molecular weight PEG. The performance was measured in the four solvents which were used for the above fouling studies: water, acetonitrile, methanol, and toluene. In this work, all of the membranes were continuously cast and there were no observable changes to the membrane morphology during grafting. Therefore, the PBI structure was consistent for all membranes and all changes to the membrane performance were exclusively due to the graft modification.

In water, the molecular weight cut off (MWCO) of the unmodified membrane was approximately 20,000 g mol⁻¹, **Fig. 6.8a**. After modification with 1kPEG, the membrane was significantly tighter, with a MWCO of approximately 4,000 g mol⁻¹. Increasing the length of the PEG modifier caused the MWCO to decrease further to 3,700 g mol⁻¹ for 2kPEG. This is believed to be because the longer graft modifiers extend further into the membrane permeation pathways, causing increased hindrance to passing solute,

and resulting in a tighter membrane. This matches previous literature on graft modification of membranes in aqueous systems [201].

However, modification with 2kPPG resulted in a similar rejection profile to the unmodified membrane. This is because of the unfavourable modifier-water interactions which caused the PPG to collapse to a smaller conformation. This resulted in no extension of the graft modifier into the membrane permeation pathways and no interaction with the permeating solute. This matched the trends of the anti-fouling properties of the modified membranes in water, with both the change in the rejection and anti-fouling properties of the modified membranes a function of the extension of the graft modifier away from the membrane surface. In water, the permeance of the modified membranes with extended graft modifiers was significantly lower, with 19, 16, 9, and 18 LMHBar for the unmodified, 1kPEG, 2kPEG, and 2kPPG respectively, shown in **Fig. 6.8e**.

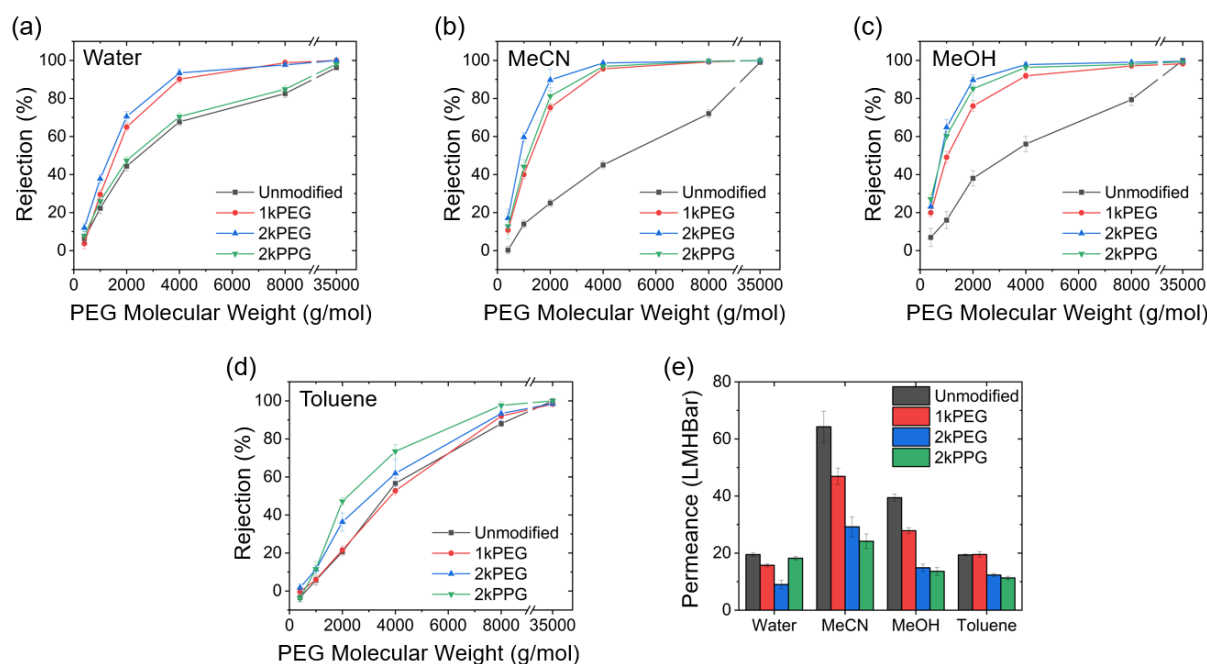


Fig. 6.8 The rejection of unmodified and graft modified PBI membranes measured using PEG of different molecular weights. The system was operated at 60 L h^{-1} cross-flow rate per cell, 30°C , 10 bar in (a) water, (b) acetonitrile (MeCN), (c) methanol (MeOH), (d) toluene. (e) The permeance of each of the membranes in each solvent.

In acetonitrile and methanol, the rejection trend after PEG modification was similar to water, shown in **Fig. 6.8b** and c. The MWCO of the unmodified membrane was approximately 20,000 g mol⁻¹ in methanol and 25,000 g mol⁻¹ in acetonitrile. In both solvents, the membrane was tighter after 1kPEG modification, and tightened further with 2kPEG modification. The rejection profile after 2kPPG modification corresponded to a rejection profile between 1kPEG and 2kPEG modification, contrary to the rejection in water. This is because 2kPPG had favourable modifier-solvent interactions in both methanol and acetonitrile, which led to the modifier extending into the permeation pathways of the membrane. Compared to the anti-fouling properties, which were closely correlated to the size of the graft modifier in solution, the change in rejection did not directly correlate to the size of the graft modifier in solution. The membrane rejection is a function of complex four-way interactions between the membrane-graft modifier-solute-solvent and these interactions result in the 2kPEG modified membrane being tighter than the 2kPPG modified membrane in polar solvents, despite the larger hydrodynamic diameter of 2kPPG in solution [9]. In methanol and acetonitrile, the permeance of the unmodified membrane was highest and decreased as the hydrodynamic diameter of the modifier increased. This showed that modifying with 2kPEG created the unexpected combination of a tighter membrane with a higher permeance than modifying with 2kPPG, which was due to the complex membrane-graft modifier-solute-solvent interactions.

In toluene, a non-polar solvent, graft modification did not change the rejection as significantly as the changes observed in polar solvents, shown in **Fig. 6.8d**. The 1kPEG modified membrane showed a similar rejection profile to the unmodified membrane, with a MWCO between 7,500 and 8,500 g mol⁻¹ for both membranes. This low change in rejection upon 1kPEG modification has been previously observed [263]. It was attributed to the collapse of the polar graft modifier to the surface of the membrane in non-polar solvents. Thus, the modifier has little interaction with passing solute and does not change the rejection profile. Modification with 2kPEG resulted in a slightly tighter membrane. This is attributed to the higher concentration of PEG on the membrane surface which resulted in a small amount of graft modifier extension away from the surface of the membrane. Increasing the hydrophobicity of the modifier, using 2kPPG, resulted in a small increase in the MWCO due to the

increase in the extension of the graft modifiers away from the surface of the membrane due to the preferential interactions with the non-polar solvent. However, in toluene there is little change in the rejection profile for all modifications. Therefore, modification with PEG and PPG give good control of the rejection profile in aqueous and polar organic solvents, but not good control in non-polar solvents.

The change in the rejection profiles after PEG graft modification was correlated to the anti-fouling properties of the membrane. The more significant the change in the rejection profile between the modified and unmodified membrane, the better anti-fouling properties the membrane possess. This is because both the rejection and anti-fouling properties are believed to be a function of the extension of the graft modifier away from the surface of the membrane, where a greater affinity of the modifier to the solvent, lead to further extension of the modifier. Therefore, the anti-fouling properties of a graft modified membrane in a desired solvent can be predicted by measuring the change in rejection after modification. This applies to the effect of the PEG based graft modification in the different solvents, however, this may not apply if the type of graft modifier is significantly changed, for example significantly changing the hydrophobicity, charge, or branching of the modifiers.

6.3.5 Effect of Pore Blocking

The anti-fouling properties of modified PBI were shown to be significantly dependant on the properties of the graft modifier. However, graft modification also led to a significant change in the rejection profile of the membranes. Therefore, one potential reason for the change in the anti-fouling properties could be due to the change in the effective pore size of the membranes. A smaller effective membrane pore size may result in less intrusion of foulant into the pores, decreasing the rate of pore blocking and fouling. Although the membranes were continuously cast with a consistent initial pore size, and no significant change in the membrane morphology was observed during modification, this hypothesis still requires investigation.

This was investigated by manufacturing a membrane with a significantly larger pore size than the pore size of the standard membranes used in this work. Therefore, after graft modification of these

membranes with the larger pore size, the effective pore size was decreased to a similar pore size to the unmodified membrane.

A membrane with larger pores was manufactured by decreasing the concentration of PBI in the dope solution from 16 wt%, which was used for all previous membranes described in this work, to 12 wt%. The 12% membrane was modified with 2kPPG using the same methodology as the 16% membrane, and the rejection profile of the 12% 2kPPG modified membrane in acetonitrile is shown in **Fig. 6.9a**. The permeance of the 12% 2kPPG membrane was 57 LMHBar, and the MWCO was $\sim 25,000$ g mol⁻¹. This permeance and selectivity were similar to the unmodified 16% membrane, which shows that the 16% unmodified membrane and the 12% 2kPPG modified membrane have a similar effective pore size.

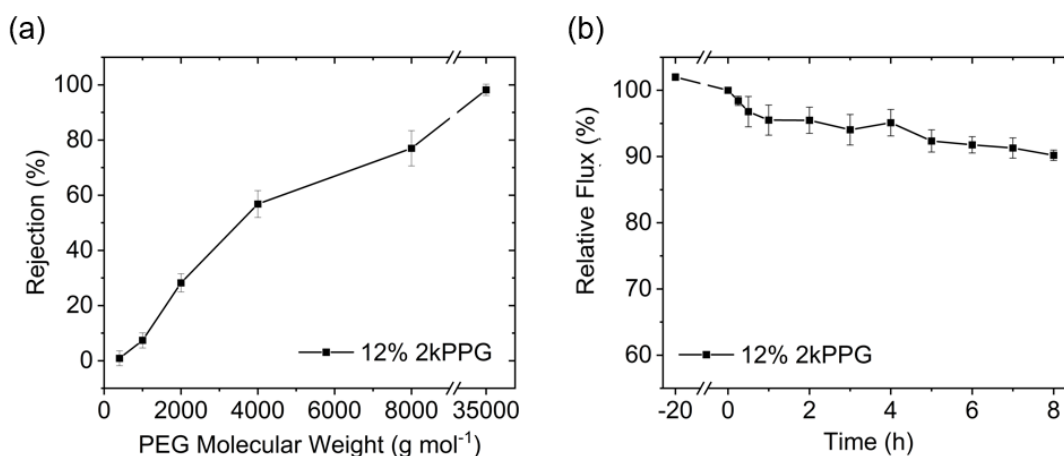


Fig. 6.9 (a) The rejection of 12% 2kPPG modified PBI membrane measured using PEG of different molecular weights. The system was operated at 60 L h⁻¹ cross-flow rate per cell, 30 °C, 10 bar in acetonitrile. (b) The relative flux of the 12% 2kPPG modified PBI membrane in acetonitrile. The initial permeance was set to approximately 200 L m⁻² h⁻¹ for each membrane and 50 ppm 20 nm silica nanoparticles were added at 0 hours. The system was operated at 60 L h⁻¹ cross-flow rate and 30 °C.

The anti-fouling properties of the 12% 2kPPG in acetonitrile are shown in **Fig. 6.9b**. Similar to the 16% membranes, the permeance was relatively constant for 20 hours prior to the addition of the colloidal silica foulant. After addition of the silica foulant, the membrane permeance decreased by 10% during eight hours continuous permeation. For the 16% membranes investigated previously in this work, the

unmodified membrane declined by 35%, and the 2kPPG modified membrane declined by 8% at the same conditions. This shows that the permeance decline of the 12% 2kPPG membrane was significantly lower than the unmodified 16% membrane with a similar rejection profile. Therefore, the antifouling effect of grafted membranes in organic solvents was attributed to the graft modification on the surface of the membrane and not due to changes in the effective pore size.

6.3.6 Other Fouling Conditions

Fouling is known to be highly dependent on the system conditions as well as the membrane properties. Because of this, the membrane fouling in acetonitrile was tested under two other experimental conditions, shown in **Fig. 6.10**. Increasing the concentration of the foulant should increase the initial rate of fouling and also the total extent of fouling. This was tested by using a ten-fold greater concentration of silica nanoparticles, increasing from 50 ppm to 500 ppm, while all other conditions were maintained, shown in **Fig. 6.10a**. With 500 ppm silica nanoparticles, the fouling of the unmodified membrane increased significantly, with the flux decreasing to just 33% of the initial flux over eight hours exposure to 500 ppm silica, compared to 66% of the initial flux with 50 ppm silica for the unmodified membrane. Furthermore, over the first 15 minutes with 500 ppm foulant, the flux had already declined by 30%. Comparatively, the 2kPPG modified membrane had a much slower initial drop in flux, with only 8% decline over the first 15 minutes, and the flux only decreased to 85% of the initial flux after eight hours with 500 ppm silica foulant. This shows that 2kPPG graft modification is more effective at high foulant concentrations and further confirms the anti-fouling benefits of PPG graft modified PBI in polar solvents.

Fouling will also change when the system's hydrodynamic conditions are changed. For example, the rate of fouling is known to increase when the cross-flow rate decreases. To check the effect on graft modified PBI, the cross-flow was decreased from 60 L h⁻¹ to 40 L h⁻¹, shown in **Fig. 6.10b**. All other conditions were maintained, and a foulant concentration of 50 ppm silica was used. It was found that the fouling increased significantly for the unmodified membrane at lower cross-flow. The final flux was 66% of the initial flux with a cross-flow rate of 60 L h⁻¹ and 48% of the initial flux with a cross-flow

rate of 40 L h^{-1} . However, the 2kPPG was less susceptible to fouling at lower cross-flow conditions, with the final flux only decreasing from 92% at 60 L h^{-1} to 88% of the initial flux at 40 L h^{-1} . This showed that the anti-fouling properties of graft modified PBI are even more effective at low cross-flow rates.

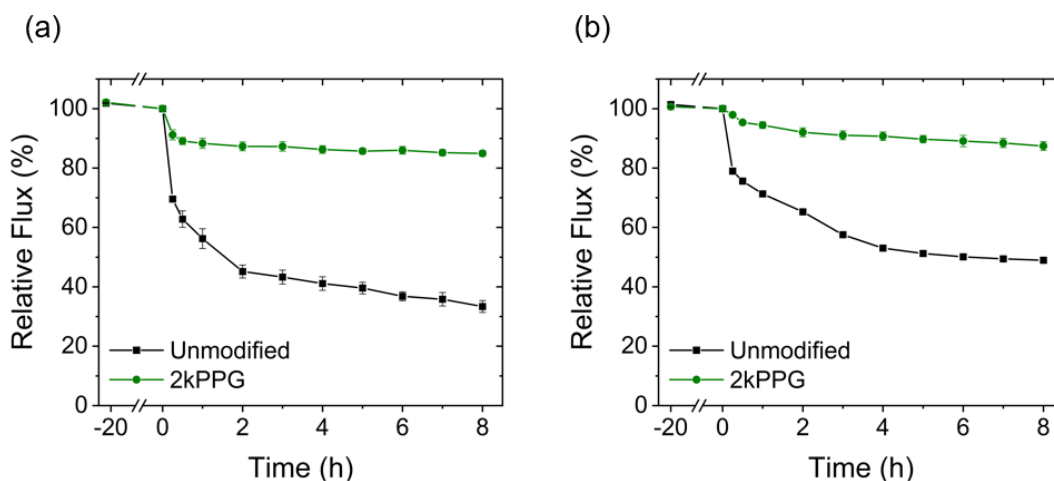


Fig. 6.10 The anti-fouling properties of the unmodified and 2kPPG modified membranes at different conditions in acetonitrile. The initial permeance was set to $200 \text{ L m}^{-2} \text{ h}^{-1}$ for each experiment and 20 nm silica nanoparticles were added at 0 hours. The system was operated at 30°C . (a) The relative flux with the silica concentration increased 10-fold to 500ppm. (b) The relative flux with the cross-flow rate decreased by 33% to 40 L h^{-1} .

These additional studies show that the improved anti-fouling properties of the graft modified membranes in polar solvents apply to a range of hydrodynamic conditions and foulant concentrations, and the results are not exclusively due to the experimental conditions used in this work.

6.4 Conclusions

Solvent stable PBI membranes were graft modified with three different modifiers, 1kPEG, 2kPEG and 2kPPG, with the aim of creating anti-fouling membranes for organic solvent separations. The anti-fouling characteristics of these membranes were tested with colloidal silica particles and were shown to be closely related to the size of the graft modifier in solution. This was attributed to a steric effect. A larger modifier increases the hindrance to the foulant reaching the surface of the membrane, and

therefore, increases the anti-fouling effect. However, the size of the modifier is significantly dependant on the solvent. In polar organic solvents, hydrophobic PPG-based modifiers were found to have a larger hydrodynamic diameter and therefore showed improved anti-fouling properties over an equivalent molecular weight PEG-based modifier. This is the opposite to aqueous systems, where PEG-based modifiers show excellent anti-fouling properties, and PPG-based modifiers show no anti-fouling benefit. Finally in non-polar solvents, both the PEG and PPG based modifiers were observed to collapse to a much smaller hydrodynamic diameter in solution, therefore providing minimal anti-fouling effect. From this work, graft modification was shown to be a good method of providing anti-fouling properties to PBI membranes. However, the choice of graft modifier needs to be carefully selected to suit the process solvent.

Chapter 7: Membrane enabled liquid phase manufacture of defined sequence polymers: pharmaceutical length oligonucleotide synthesis

Abstract

Membrane enabled liquid phase synthesis is proposed as a new manufacturing platform for defined sequence polymers (DSPs). This platform technology aims to improve on the current industrial standard for DSP manufacture, solid phase synthesis (SPS), by combining the fast and scalable reactions of liquid phase synthesis (LPS), with high purity and high yield purifications using organic solvent stable membrane separations.

In this work, the first pharmaceutical length oligonucleotide was synthesised using membrane enabled liquid phase synthesis. A custom ‘synthesiser’ was designed to ensure that the process was performed in a single apparatus with no material transfer. This enables the one-pot oligonucleotide synthesis to be fast and fully automated, comparable to the current industrial SPS process. A 20-mer oligonucleotide was manufactured in the synthesiser on a 4-arm 40 kDa PEG hub molecule, which increased the oligonucleotide solubility and enabled purification exclusively with organic solvent-stable polybenzimidazole membrane separations. The final oligonucleotide was synthesised at tens of gram scale and had a good crude purity (75.2%) and an average yield (50%), which were comparable to SPS. This proof-of-concept study showed the significant potential of membrane enabled liquid phase synthesis for oligonucleotide manufacture. With the potential advantages of scalability, economies of scale, and removal of expensive solid phase resins, this technology may surpass SPS in the future.

Keywords: Liquid phase synthesis (LPS), Oligonucleotide, Organic Solvent Ultrafiltration (OSU), Polybenzimidazole (PBI), One pot synthesis

Highlights

- A one-pot, single phase synthesiser was designed for oligonucleotide manufacture
- A 4-arm 40 kDa soluble hub was optimised to have high rejection with PBI membranes
- A 20-mer oligonucleotide was synthesised on this hub in liquid phase at tens of gram scale
- The protected 20-mer was purified exclusively by membrane separations
- The final 20-mer oligonucleotide was manufactured at 75.2% crude purity and 50% yield

Acknowledgements

The author kindly thanks Exactmer Ltd. for designing and optimising the chemistry of oligonucleotide synthesis which is shown in **Fig. 7.2**. And for analysing the globally deprotected oligonucleotide, which is shown in **Fig. 7.10**.

7.1 Introduction

Defined sequence polymers (DSPs) have a fully defined primary structure. This means they have an exact chain length and a precisely ordered sequence of monomers [17,34]. Due to this, DSPs exhibit unique material properties and have applications in materials science [34], drug-delivery [37], data storage [38], anti-counterfeiting technologies [39], catalysis [40], protein folding [41], and optoelectronic devices [42]. Additionally, DSPs are commonly used as pharmaceuticals, with defined sequence peptides and oligonucleotides providing highly effective treatments to a wide range of diseases [47,57].

Industrial manufacture of peptides and oligonucleotides predominantly uses solid phase synthesis (SPS), which is described in detail in **Chapter 2**. SPS is a highly flexible manufacturing technique which facilitates rapid purifications and highly automated synthesis. However, this technique has limitations due to high operating cost, difficult inline monitoring, difficult scale up, and mass transfer limited reactions [4,330–332].

Due to these disadvantages, liquid phase synthesis (LPS) is often proposed as an alternative method to SPS. Performing the reactions in a liquid phase promotes fast reactions with no mass transfer limitations, easy inline monitoring, and scalability. However, the purification of the DSP from excess reagents and reaction debris is a significant challenge. Chromatography, extraction, and precipitation have been proposed as purification techniques for LPS. However, these techniques are time consuming, difficult to automate, and struggle to achieve high purities and yields [109,111,115,116]. Therefore, although LPS shows significant benefits over SPS, the difficult separations of LPS result in SPS dominating the industrial market [24].

Membrane separations in organic solvents are an alternative purification technique for LPS. Organic solvent nanofiltration (OSN) and organic solvent ultrafiltration (OSU) are a relatively new field, but these technologies enable high purity, high yield separations, all in one single phase [8,11]. Combining OSN or OSU to LPS has the potential to generate a fully automatable and scalable process which could

surpass industrial SPS. To date, membrane enabled liquid phase DSP synthesis has been used for the synthesis of three classes of DSP: peptides, poly(ethylene glycol), and oligonucleotides.

So et al. synthesised two different pentapeptides on a 5 kDa PEG support [121]. Yields of 54.6% and 82% were obtained for the two different peptide sequences. However, the membrane showed high amino acid rejection which resulted in slow purifications, and every purification required a large quantity of solvent. Dong et al. used membrane enabled iterative synthesis to manufacture a functionalised poly(ethylene glycol) hexamer on a three-armed benzene-based support [5]. A two-stage membrane system was required, resulting in an overall yield of 82.2%. However, there was constant material transfer between reactions in a glass flask and separations in a diafiltration rig, which makes the process difficult to automate and scale up. Gaffney et al. and Kim et al. synthesised a 9-mer oligonucleotide on a three-arm benzene-based support, which is the longest published DSP using membrane separations [7,29]. They achieved 49% crude purity, which was in part due to poor separations, with traces of monomer remaining after each cycle. They also achieved a relatively low yield (39%), due to significant permeation of the oligonucleotide through the membrane, with rejections of the oligonucleotide between 95% and 99.5%.

These studies showed the potential of membrane enabled liquid phase DSP synthesis with this technique successfully synthesising DSPs up to 9-mer. However, for pharmaceutical DSPs, longer sequences are required. In previous studies, the maximum length of the DSP manufactured through membrane enabled DSP synthesis was restricted due to permeation of the soluble support through the membrane. This was due to limitations of the membranes and resulted in the yield continuously declining throughout the synthesis. However, due to the advancement of solvent stable membranes detailed in **Chapter 4 to 6** of this work, it is predicted that long sequence DSPs can be manufactured at high yield through membrane enabled liquid phase synthesis using PBI membranes.

In this work, an oligonucleotide DSP was selected to show the potential of membrane enabled DSP synthesis. Therapeutic oligonucleotides are medium length sequences, often around 18 to 25 monomers in length, which show highly specific and selective pharmaceutical properties [13,20,98,333]. There are

currently 15 FDA approved oligonucleotide therapeutics, two of which have a high market demand, Inclisiran and Pegaptanib [54–56]. To meet the large-scale demand of these drugs, a scalable method of manufacture, such as LPS, is critical.

This work focused on the manufacture of a pharmaceutical length oligonucleotide of 20 monomers. The primary target was to achieve a purity and yield which is comparable to SPS. The secondary targets were to achieve this in a fully automatable one-pot process, with no material transfer, and to exclusively purify the 20-mer protected oligonucleotide using membrane separations.

7.2 Material and Methods

7.2.1 Materials and Equipment

For membrane testing, HPLC grade acetonitrile was purchased from VWR. 2,4-dinitro-1-naphthol (Martius Yellow), sucrose octaacetate (SoA), and polyethylene glycol (10000, 20000, and 35000 g mol⁻¹) were purchased from Sigma Aldrich. Methoxy PEG 10 kDa amine, 4-arm 10 kDa PEG amine, and 8-arm 10 kDa PEG amine were purchased from Jenkem Technology.

For membrane manufacture, a custom solution of PBI (1.1 IV powder dissolved at 18 wt% with 1.5 wt% lithium chloride in N,N-dimethylacetamide) was purchased from PBI Performance Products. Novatexx 2471 non-woven support, made of polypropylene, was purchased from Freudenberg Filtration Technologies. HPLC grade acetonitrile (MeCN) and propan-2-ol (IPA) was purchased from VWR. α,α' -Dibromo-*p*-xylene (DBX), N,N-dimethylacetamide, N,N-diisopropylethylamine, 2-methoxyethylamine, acetic anhydride, 4-dimethylaminopyridine, and PEG (400 g mol⁻¹) for membrane synthesis were purchased from Sigma Aldrich. The graft modifier M-2005 (2kPPG) was purchased from Huntsman.

For oligonucleotide synthesis, DMT-2'-O-Me-C(Ac)-CE phosphoramidite, DMT-2'-O-Me-A(Bz)-CE phosphoramidite, N³-Bz-DMT-2'-O-Me-U-CE phosphoramidite, and DMT-2'-O-Me-G(i-Bu)-CE phosphoramidite were purchased from Hongene. Cyanoethanol (CNeOH), xanthane hydride (XH), dicyanoimidazole (DCI), pyridine, and trifluoroacetic acid (TFA) were purchased from TCI. 4 Arm-PEG 40 kDa-sarcosine-succinate-mU-DMTr and a proprietary thiol-based cation trap were kindly prepared

by Exactmer Ltd, using proprietary techniques. Diethylamine and ammonia, for global deprotection were purchased from Sigma Aldrich. HPLC grade acetonitrile and N,N dimethylformamide (DMF) were purchased from VWR.

The synthesiser was manufactured using a 0.5 L glass tank (QVF), a diaphragm pump (G03, Wanner), five 51 cm² circular membrane cells, and a back pressure regulator (Equilibar). The temperature was controlled using a heat exchanger (made with Swagelok tubing and a Lauda chiller). The synthesiser was monitored using level sensors (LFP Inox, Sick), flow and temperature sensors (RHM 04, Rheonik), pressure sensors (StrainSense), and inline UV monitoring (Uniqsis).

7.2.2 Methods

7.2.2.1 Synthesiser Design and Development

To investigate the mixing pattern inside the synthesiser, an inline UV sensor (Uniqsis) was placed on the retentate line, as shown in **Fig 7.4b**. Five circular cross-flow cells of 51 cm² each were used in series and the permeate line of each cell was closed with a valve. The rig was filled with acetonitrile and operated with constant recirculation, at 2 kg min⁻¹, 1 kg min⁻¹, or 0.6 kg min⁻¹, 30 °C, and minimum pressure. 1 mL of dilute dye (10 g L⁻¹ martius yellow dye in acetonitrile) was rapidly injected into the feed tank. Simultaneous to injection, the retentate line was opened to a waste tank and the synthesiser was switched to single pass mode, as shown in **Fig 7.4b**. To maintain a constant system volume, fresh acetonitrile was added to the feed tank at the pump flow rate. The UV output of the dye injections was measured at each flow rate three times.

For measuring the effect of injection speed, the synthesiser was filled with acetonitrile and operated in recirculation mode, at 0.6 kg min⁻¹, as shown in **Fig. 7.4b**. For fast injections, 1 mL of dilute dye (10 g L⁻¹ martius yellow dye in acetonitrile) was rapidly injected into the feed tank. For slow injections, 1 mL of dilute dye (10 g L⁻¹ martius yellow dye in acetonitrile) was slowly injected over one system residence time using a syringe pump (Dissolution Accessories). The UV signal was monitored throughout, and these experiments were repeated three times.

7.2.2.2 Hub Optimization

Hub optimisation experiments used two 51 cm² cross-flow cells of M-97.5 membrane from **Chapter 4**. A 400 mL test rig was operated at 2 kg min⁻¹ and 30 °C. The membranes were compacted in acetonitrile at 20 bar for a minimum of 24 h. This duration was shown to give constant permeance after compaction. Following this, the pure solvent permeance was measured at 10 bar, 15 bar, and 20 bar. Then SoA was added to a concentration of 10 g L⁻¹ SoA in acetonitrile, and the rejection and permeance were measured at the same three pressures. Incremental amounts of either PEG 10 kDa, PEG 20 kDa, or PEG 35 kDa were dissolved in the minimum volume of 10 g L⁻¹ SoA in acetonitrile and added. The rejection and permeance was measured at the three pressures and increasing concentration until 50 g L⁻¹ PEG was present in the system. The rejection was measured using HPLC, with the method described in **Section 7.2.2.6**.

The rig was then washed with pure acetonitrile and the pure solvent permeance measured. For all experiments, there was less than 5% flux decline, showing that the membranes had not changed significantly throughout the duration of the experiment.

The same rig was used for central composite design (CCD) optimisation. Two 51 cm² discs of M-97.5 membrane from **Chapter 4** were compacted in acetonitrile for a minimum of 24 hours at 20 bar and 30 °C. The rig was operated with 25 g L⁻¹ of methoxy PEG 10 kDa amine, 4-arm 10 kDa PEG amine, or 8-arm 10 kDa PEG amine in acetonitrile. The system was pressurised between 2 bar and 12 bar and a cross-flow rate between 0.75 kg min⁻¹ and 2 kg min⁻¹ was used, at the values shown in **Fig. 7.6a**, in a randomised order. For each experimental condition, the permeance was measured and the rejection was determined using HPLC, with the method described in **Section 7.2.2.6**.

The rig was also washed with pure acetonitrile and the pure solvent permeance measured after the experiment was completed. For this experiment, there was also less than 5% flux decline

7.2.2.3 Membrane Screening

Membrane screening was performed with four different membrane types. These were 14 wt% high molecular weight PBI modified with 2-MEA and 2kPPG (14_2-MEA and 14_2kPPG respectively), and

18 wt% high molecular weight PBI modified with 2-MEA and 2kPPG (18_2-MEA and 18_2kPPG respectively).

These membranes were prepared using the continuous casting methods described in **Chapter 6**. The 14% PBI was cast at 200 μm and the 18% PBI was cast at 150 μm . A 3.2 m x 0.2 m sheet of membrane was crosslinked in a 2 L reactor with 0.08 mol L⁻¹ DBX dissolved in acetonitrile. The crosslinking reaction was continuously recirculated with a gear pump (GD series, Micropump) and maintained at 70 °C for 24 h under an argon atmosphere. After crosslinking the membranes were washed with fresh acetonitrile.

For the 2kPPG membranes, the membranes were modified after crosslinking by immersion in acetonitrile containing a hindered base, N,N-diisopropylethylamine (0.6 mol L⁻¹), and the graft modifier 2kPPG (0.2 mol L⁻¹). The modification reaction was maintained at 70 °C under an argon atmosphere. After 24 h 2-methoxyethylamine (0.2 mol L⁻¹) was added and maintained at 70 °C under an argon atmosphere for a further 24 h. After modification, the membranes were washed with fresh acetonitrile.

For the 2-MEA membranes, the membranes were modified after crosslinking by immersion in acetonitrile containing a hindered base, N,N-diisopropylethylamine (0.6 mol L⁻¹), and the capping agent 2-methoxyethylamine (0.2 mol L⁻¹). The modification reaction was maintained at 70 °C under an argon atmosphere for 24 h. After modification, the membranes were washed with fresh acetonitrile.

After modification, both the 2kPPG and 2-MEA membranes were passivated. The membranes were immersed in acetonitrile containing acetic anhydride (0.24 mol L⁻¹), N,N-diisopropylethylamine (0.5 mol L⁻¹), and 4-dimethylaminopyridine (0.006 mol L⁻¹). The reaction was maintained at room temperature under an argon atmosphere for 4 h. After passivation, the membranes were washed with fresh acetonitrile, followed by washing with IPA.

After washing, the membranes were immersed in a mixed solution of PEG (400 g mol⁻¹)-IPA 1:1 (v/v) for 24 hours at room temperature with constant stirring. The membrane was then removed from the solution and dried at room temperature in a fume cupboard.

These membranes were inserted into 51 cm² cross-flow cells and tested in the synthesiser, shown in **Fig. 7.3**. 100 g L⁻¹ 4 Arm-PEG 40 kDa-sarcosine-succinate-mU-DMTr in acetonitrile was added. The rig was operated at 2 kg min⁻¹ cross-flow, 30 °C, and 10 bar transmembrane pressure. The permeance was taken and the rejection of a minimum of four membrane discs was measured by UHPLC.

7.2.2.4 Fouling

The fouling of the membranes in the presence of the proprietary thiol-based cation trap was tested. The pure acetonitrile permeance of a PBI-18_2MEA was measured in a 12 cm² dead end cell at 30 °C, 10 bar. After the pure solvent tests, 1000 mL thiol-based cation trap was filtered through the membrane at the concentration used in the synthesiser at 30 °C, 10 bar. After this, the pure acetonitrile permeance measurement was repeated at 30 °C, 10 bar.

7.2.2.5 Oligonucleotide Synthesis

For oligonucleotide synthesis, 4 Arm-PEG 40 kDa-sarcosine-succinate-mU-DMTr (40 g) was dissolved in dry acetonitrile and filtered through a 100 nm PTFE filter. This was loaded into a synthesiser containing acetonitrile (400 mL), shown in **Fig. 7.3**. Five 51 cm² cross-flow cells of PBI 18_2-MEA were used. All reagents which were added to the synthesiser were added slowly over one system residence time. The first step of the synthesis was diafiltration 1.

Chain Extension

For the chain extension, the synthesiser was operated at 30 °C, 0.6 kg min⁻¹. The back pressure regulator was operated at the minimum pressure and a valve was closed on the membrane permeate line. Building block (1.5 eq. per arm) was dissolved in dry acetonitrile (20mL), filtered (200 nm PTFE filter), and added to the synthesiser. The building blocks used were DMT-2'-O-Me-C (Ac)-CED phosphoramidite, DMT-2'-O-Me-A(Bz)-CED phosphoramidite, N3-Bz-DMT-2'-O-Me-U-CED phosphoramidite, or DMT-2'-O-Me-G (i-Bu) CED phosphoramidite, in the order shown in **Fig 7.1a**. After two minutes, DCI (1.83 g, 4 eq. per arm) dissolved in dry acetonitrile (15 mL) was filtered through a PTFE filter (200 nm) and added to the synthesiser to initiate the chain extension.

After 20 minutes CNeOH (0.83 mL, 3 eq. per arm) dissolved in dry acetonitrile (15 mL) was filtered through a PTFE filter (200 nm) and added to the synthesiser to quench the chain extension reaction. After a further two minutes, XH (1.75 g, 3 eq. per arm) dissolved in pyridine (16 mL) was filtered through a PTFE filter (200 nm) and added to the synthesiser to thiolate the phosphoramidite. After five minutes, diafiltration 1 was started.

Diafiltration 1 (DF1)

For DF1, the synthesiser was pressurised to 10 bar, the cross-flow rate increased to 2 kg min^{-1} , and the valve on the permeate line of the cells was opened to a permeate collection tank. Four system volumes (1.6 L) were permeated, and the system was maintained at a constant volume with continuous addition of fresh dry acetonitrile. The diafiltration temperature was 30°C for the first 10 chain extensions, 35°C for the following 9 chain extensions, and 40°C for the final chain extension. The temperature was increased to decrease the solution viscosity and increase the rate of diafiltration. After DF1, detritylation was started.

Detritylation

For detritylation, the flow rate was decreased to 0.6 kg min^{-1} and the synthesiser was depressurised. The back pressure regulator was operated at the minimum pressure and a valve was closed on the permeate line. A thiol-based cation trap (5 eq. per arm) dissolved in acetonitrile (10 mL) was filtered through a PTFE filter (200 nm) and added to the synthesiser. After two minutes of recirculation, TFA (15 mL) was added to the synthesiser to deprotect the oligonucleotide. After 20 minutes, pyridine (40 mL) was added to the synthesiser to quench the TFA. After five minutes diafiltration 2 was started.

Diafiltration 2 (DF2)

For DF2, the synthesiser was pressurised to 10 bar, the cross-flow rate increased to 2 kg min^{-1} , and the temperature was changed to the same temperature as DF1. A valve on the permeate line of the cells was opened to a permeate collection tank and a minimum of six system volumes were permeated (2.4 L). The system was maintained at a constant volume with addition of fresh dry acetonitrile. After permeation of six system volumes, the remaining building block in the synthesiser was checked. If this

was not < 1% of the initial unreacted building block, diafiltration was continued until this condition was achieved. After DF2, the next chain extension was started with the following building block in the sequence, shown in **Fig. 7.1a**.

Global Deprotection

After 20 cycles, the full-length oligonucleotide was washed out of the synthesiser using acetonitrile and globally deprotected. The crude oligonucleotide was dissolved in the minimum amount of DMF, and dry diethylamine 30% (v/v) was added. After 30 minutes, the DMF was evaporated, and the residue dissolved in water-ammonia 3:1 (v/v). This was heated at 55 °C overnight. The next day, the ammonia was evaporated, and the residue dissolved in acetonitrile and centrifuged (5 minutes 600 rpm). The precipitate was repeatedly washed and centrifuged before it was analysed using UHPLC-MS, with the method described in **Section 7.2.2.6**.

7.2.2.6 Analytical Methods

Rejection samples were analysed using the HPLC method described in **Section 4.2.2.8**.

Membrane screening and oligonucleotide synthesis samples were analysed using an Agilent UHPLC. An Acquity UPLC BEH C18 column was used, eluting with a gradient of two mobile phases: Mobile phase A was 0.1 % formic acid aqueous solution; mobile phase B was acetonitrile-methanol 4:1 (v/v). A linear ramp from 20 % to 100 % B was used, with a flow rate of 0.4 mL min⁻¹ and a column temperature of 30 °C.

The globally deprotected oligonucleotide was analysed with a UHPLC-MS (Agilent 1290 Infinity II). An Acquity UPLC Peptide C18 column was used, eluting with a gradient of two mobile phases: Mobile phase A was methanol-water 1:9 (v/v) with 2 µM ethylenediaminetetraacetic acid, 60 mM hexafluoro-2-propanol, and 6 mM hexylamine; mobile phase B was acetonitrile-methanol 1:1 (v/v). A linear ramp from 20 % to 40 % B was used, with a flow rate of 0.4 mL min⁻¹ and a column temperature of 65 °C.

7.3 Results and Discussion

7.3.1 Synthesis Strategy

A 2'-methyl RNA phosphorothioate oligonucleotide was selected to develop the process of membrane enabled liquid phase DSP synthesis. This oligonucleotide sequence was M23D (+2-18), shown in **Fig. 7.1a-c** [334]. It is of pharmaceutically relevant length, 20-mer, and has been used previously to test new techniques for manufacturing oligonucleotides [7,47]. To manufacture the target oligonucleotide, the iterative synthesis strategy originally developed by Kim et al. [29] was used, which combined liquid phase reactions with membrane separations, shown in **Fig. 7.1d**, and described in detail in **Chapter 2**. The first step of the synthesis strategy was the attachment of a soluble hub molecule to the 3' end of the first oligonucleotide base. Then a single oligonucleotide monomer was added to the 5' end of the oligonucleotide base, using a four-step process of chain extension, diafiltration one (DF1), deprotection, diafiltration two (DF2). This four-step process was iteratively repeated 'n' times and each iteration added one monomer to the oligonucleotide sequence, with 'n' corresponding to the number of monomers in the final DSP.

The iterative cycle consisted of four key steps. These steps are summarised in **Table 7.1**. The first synthetic step of the iterative cycle was the chain extension of the oligonucleotide. The chain extension added the 'n+1' protected phosphoramidite onto the 5'OH of the oligonucleotide sequence of 'n' bases, increasing the length of the growing oligonucleotide by a single monomer, shown in **Fig. 7.2a**. After the chain was extended the reaction was quenched and the internucleotide linkage thiolated. The chemistry of these steps is well described in detail in literature [7,29,332,335].

Following the chain extension, the second step of the cycle was to purify the growing oligonucleotide. This was achieved using a constant volume diafiltration with organic solvent stable PBI membranes. The diafiltration was performed in the solvent used for the chain extension reaction to remove any requirements for solvent exchange, which is time consuming and solvent intensive. The first diafiltration (DF1) removes the unreacted monomer and reaction debris from the growing

oligonucleotide, specifically removing the pyridine from the system, which has a significant negative impact on the next step of the cycle.

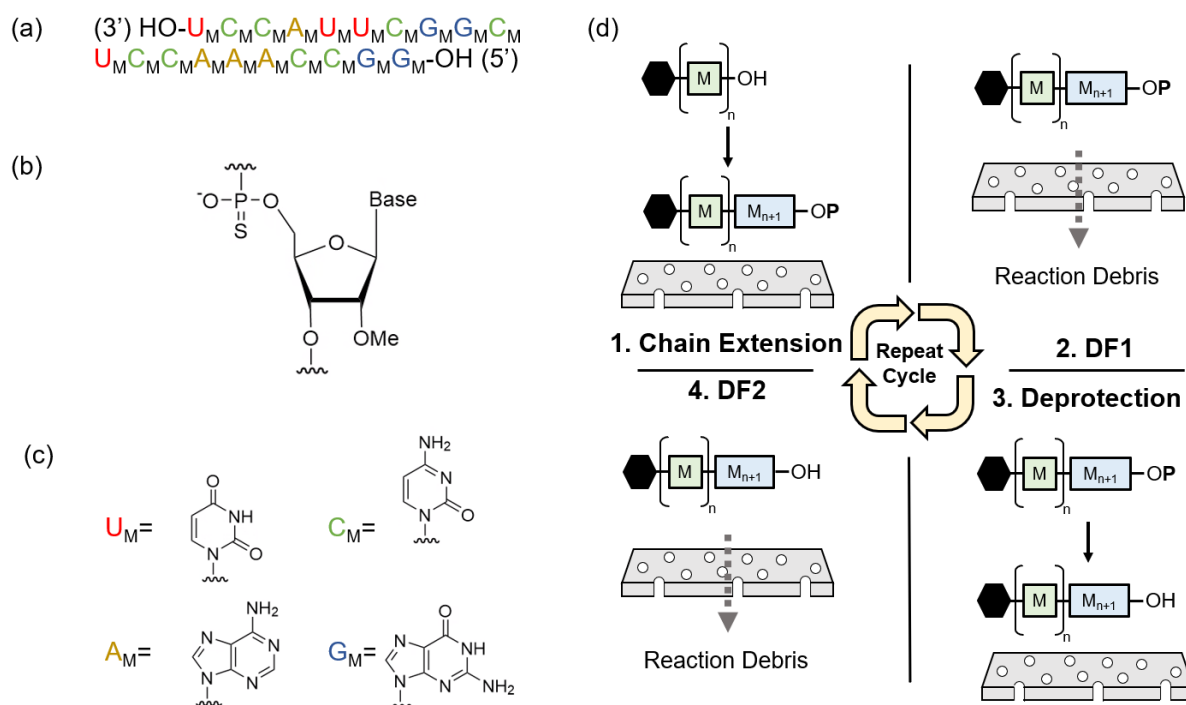


Fig. 7.1 (a) M23D (+2-18), the target 20 monomer oligonucleotide sequence. (b) The structure of each monomer of M23D (+2-18), where the structure of the bases: U_M , C_M , A_M , or G_M is shown in (c). (d) The proposed synthesis cycle, where each complete cycle adds one monomer to the growing oligonucleotide. Each cycle consists of 1. Chain extension, 2. DF1, 3. Deprotection, and 4. DF2. The black hexagon denotes a soluble hub molecule, and the P denotes a protecting group.

All previous work on membrane enabled DSP synthesis performed the reactions in a reaction flask and relied on material transfer into and out of the diafiltration apparatus to perform the purification [7,29]. This is feasible for short sequences, however, for the synthesis of a 20-mer oligonucleotide, this equates to 40 material transfer steps into the diafiltration apparatus, and a further 40 material transfer steps out of the diafiltration apparatus. Every material transfer step requires a large volume of solvent to wash the diafiltration apparatus, and subsequent evaporation of the solvent to recover the growing oligonucleotide. This process is time consuming, energy intensive, and difficult to achieve 100% yield,

automate, and scale. Therefore, this study aimed to improve on the previous work by designing a one-pot system which required no material transfer.

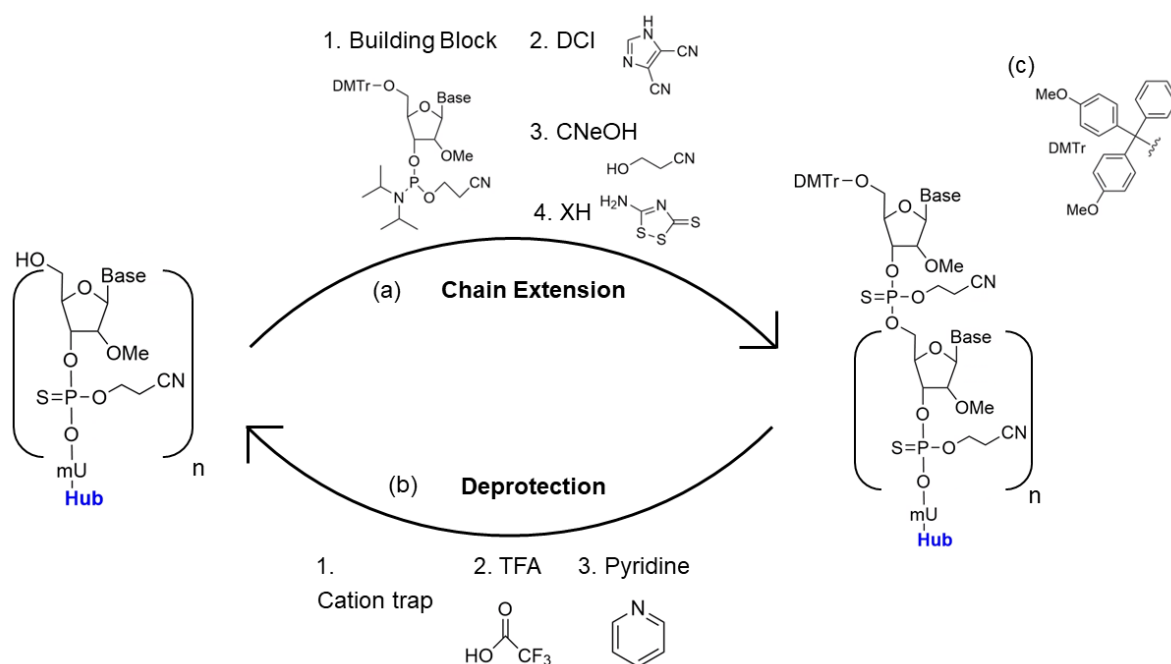
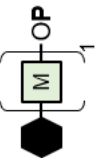
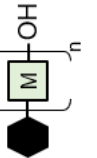
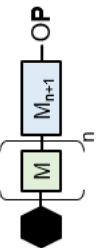
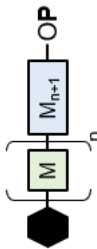
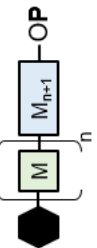
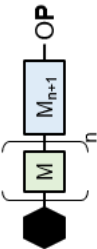
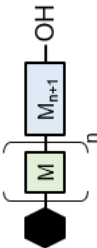
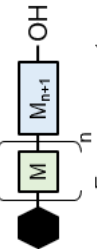
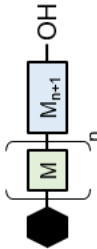


Fig. 7.2 (a) The chain extension reaction to manufacture M23D (+2-18), consisting of 1. Addition of building block (BB), 2. Activation with DCI, 3. Quench with CNeOH, 4. Sulphur transfer with XH. (b) The deprotection reaction to manufacture M23D (+2-18), consisting of 1. Addition of a thiol-based cation trap, 2. Deprotection with acid, TFA, 3. Quench with base, pyridine. (c) The structure of the DMTr protecting group. This chemistry was designed by Exactmer Ltd.

This was achieved by adding the reagents for the chain extension reaction to the retentate loop of the diafiltration rig and performing the chain extension inside the retentate loop at low pressure. Because the reactions were performed in the retentate of the diafiltration rig, switching from the chain extension reaction to the diafiltration simply required pressurisation of the system. Although this added significant complications to the engineering of the diafiltration rig, discussed in **Section 7.3.2**, this improvement removed all material transfer steps, which were required in all previous membrane enabled DSP syntheses. This improvement enabled the entire synthesis of the oligonucleotide to be performed in one single apparatus, which promotes high yields, scalability, and automation. From here, the rig which was used for both the reactions and diafiltrations is referred to as a ‘synthesiser’, shown in **Fig. 7.3**.

Table 7.1 The loading, four step synthetic cycle, and unloading step for membrane-based oligonucleotide synthesis. The four synthetic steps are repeated ‘n’ times.

Operation Step	Reagents added to the synthesiser	Reagents removed from the synthesiser	Reaction time/ Volume of permeate	Flow rate (kg min ⁻¹)	Pressure (Bar)	Synthesiser contents: Start of operation step	Synthesiser contents: End of operation step
Synthesiser Loading	Hub-M ₁	-	-	-	-	Pure Acetonitrile	
Chain Extension	Building block	-	2 min	0.6	0		
	DCI	-	20 min	0.6	0		+ Excess reagents + Reaction debris
	CNeOH	-	2 min	0.6	0		
	XH in pyridine	-	5 min	0.6	0		
Diafiltration 1	Acetonitrile	-					
	Building block	-					
	DCI	-	1.6 L	2	10		+ Excess reagents + Reaction debris
	CNeOH	-					
Detritylation	XH	-					
	Pyridine	-					+ Excess reagents + Reaction debris
	Cation trap	-	2 min	0.6	0		
Diafiltration 2	TFA	-	20 min	0.6	0		
	Pyridine	-	5 min	0.6	0		+ Excess reagents + Reaction debris
	Acetonitrile	-					
	Cation trap - DMTr	-	2.4 L	2	10		+ Excess reagents + Reaction debris
Synthesiser Unloading	Acetonitrile	-					Pure Acetonitrile
	Hub-oligonucleotide	-					

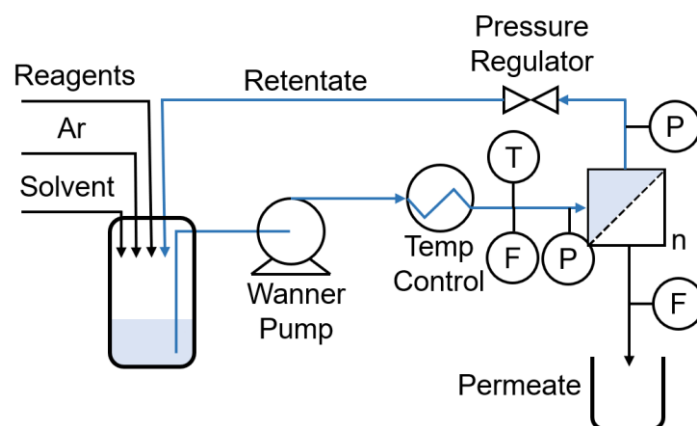


Fig. 7.3 The oligonucleotide synthesiser designed and constructed to perform the synthesis of M23D (+2-18), where $n = 5$ cells in series. All chain extension and deprotection reactions were performed inside the retentate loop of the synthesiser, shown in blue, with constant recirculation at 0.6 kg min^{-1} .

When complete, the DF1 was stopped by simply depressurising the system. After DF1, the ‘ $n+1$ ’ protected oligonucleotide was deprotected, shown in **Fig. 7.2b**. The chemistry of this step is described in detail in literature [7,29,332,335]. Similar to the chain extension, all reagents for the removal of the trityl protecting group, shown in **Fig. 7.2c**, were added directly to the synthesiser and the reactions were completed inside the retentate loop at low pressure.

After deprotection, the synthesiser was repressurised and a second constant volume diafiltration (DF2) was completed to fully remove any remaining unreacted building block, protecting group debris, and salts. The end point for DF2 was when only the purified growing oligonucleotide remained in the synthesiser. When DF2 was complete, the synthesiser was depressurised, and the four-step cyclic process was repeated using the next monomer in the sequence. For the synthesis of M23D (+2-18), this cyclic process was repeated 20 times. After 20 cycles, the oligonucleotide was washed out of the synthesiser. The 3’ end of the oligonucleotide was then cleaved from the hub and the oligonucleotide was fully deprotected. The final oligonucleotide was then purified and characterised.

7.3.2 Synthesiser Design

In the synthesis of a DSP, there are a number of common impurities which need to be minimised in the final product. Two of the most important impurities are deletion impurities, such as ‘ $n-1$ ’, and addition

impurities, such as 'n+1'. Here, 'n' represents the full-length sequence, with the minus one representing the sequence missing one single monomer, and the plus one representing a sequence with the addition of one single monomer. Due to the nature of iterative synthesis, the formation of a small quantity of n-1 or n+1 in every iteration results in a significant decrease of the final full-length product purity [332,336].

N-1 impurities will form if any reaction is incomplete. These impurities are often formed in solid phase synthesis reactions due to diffusional limitations and channelling inside the solid resin, which makes it difficult to achieve 100% reaction completion. Conversely, for liquid phase reactions in a reaction vessel, the mixing is efficient and coupling efficiencies up to 99.95% are regularly achieved [7,25,332]. Performing these liquid phase reactions in a synthesiser, instead of a reaction vessel, results in the mixing inside the synthesiser having a significant effect on the quantity of n-1 formed.

If any unmixed zones or bypass zones are present in the synthesiser, the reactants are slow to enter these regions during the short reaction time. This results in no reaction occurring in these zones, which produces an n-1 impurity. However, during the longer diafiltrations, any n-1 impurities in the unmixed zones or bypass zones diffuse back into the bulk of the synthesiser. This means that performing the reactions in a single apparatus DSP synthesiser requires specific design considerations to ensure that the synthesiser is well mixed.

The theoretical effect of different volumes of unmixed zones is shown in **Fig. 7.4a**. If 1 mL of the synthesiser is completely unmixed, and the reactants cannot enter this region during the reactions, the maximum achievable purity of the final oligonucleotide is 90%. If this unmixed volume is increased to 5 mL or 10 mL, the maximum achievable purity is decreased to 60% and 36% respectively. Therefore, removal of all poor mixed zones is critically important to achieve a high purity product.

To remove unmixed zones, hygienic engineering design principles were followed to select the equipment. The basic synthesiser design followed the set up used by Kim et al. [29]. However, in the previous work, the equipment was only used for separation, where unmixed zones are not a significant problem. Therefore, based on guidelines from the European Hygienic Engineering and Design Group,

the equipment, including the pressure regulator, sensors, pump, and feed tank, was upgraded to meet the recommended hygienic design criteria. An example of the hygienic design principles was an upgrade of the pressure sensor. Kim et al. used a Wika analog pressure gauge in their system [29]. However, this sensor contained ~1 mL volume in a dead end between the sensor and the t-piece where it was mounted into the process. The length of this dead zone was greater than the diameter of the pipe. Therefore, based on the guidelines from the European Hygienic Engineering and Design Group, this must not be present in the synthesiser. Because of this, the gauge was changed to a flush mounted pressure gauge from Strainsense, which met the design criteria. This change removed ~1 mL dead volume and will increase the final oligonucleotide purity by up to 10%. The final synthesiser design is shown in **Fig. 7.3**, with each component detailed in **Section 7.2.1**.

In addition to theoretically identifying and removing regions of poor mixing, the mixing pattern of the synthesiser was characterised using tracer experiments. This work was essential as the mixing pattern inside the circular membrane housing needs to be fully characterised. An inline UV sensor was placed downstream of the membrane cells and a series of dye injections into the inlet of the pump were performed. The synthesiser was operated in single pass mode, using the equipment shown in **Fig. 7.4b**. At a flow rate through the synthesiser of 0.6 kg min^{-1} (the flow rate used for the reactions), and with an instantaneous dye injection at $t = 0 \text{ s}$, the maximum dye concentration was observed at a time equal to the mean residence time (τ), shown in **Fig. 7.4c**. This showed that there was no significant bypass in the flow pattern of the synthesiser, which is essential for ensuring that all reagents are well mixed inside the synthesiser and reducing the formation of $n-1$ impurities. Additionally, this was shown to be independent of the system flow rate, between 0.6 kg min^{-1} and 2 kg min^{-1} , which encompasses the range of flowrates used in the reactions and diafiltrations.

Qualitatively, the presence and shape of the peak tail in the dye experiments showed that the synthesiser had predominantly plug flow reactor (PFR) behaviour. This was quantified using the Peclet number. At 0.6 kg min^{-1} the Peclet number was ~18. A high Peclet number corresponds to PFR behaviour, and a low Peclet number corresponds to continuous stirred tank reactor (CSTR) behaviour [337,338].

Therefore, the synthesiser had predominantly PFR behaviour at the flow rate used in the reactions. This behaviour makes it difficult to ensure that the reagents are fully mixed throughout the synthesiser.

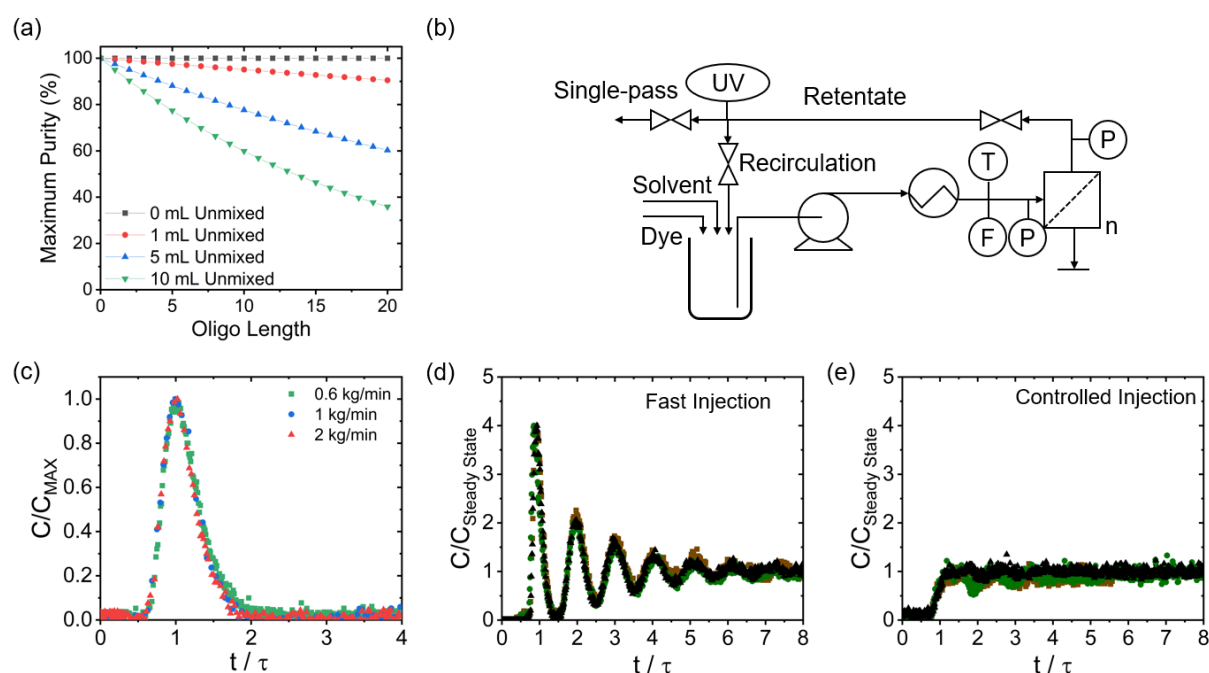


Fig. 7.4 (a) The maximum achievable purity for the synthesis of different length oligonucleotides with an increasing volume of unmixed solution inside the oligonucleotide synthesiser. (b) The synthesiser with UV monitoring used to analyse mixing patterns and bypass flow, where $n = 5$ cells in series. (c) The UV signal of fast injection of dye into the pump inlet at 0.6 kg min^{-1} , 1.0 kg min^{-1} , and 2.0 kg min^{-1} in single-pass synthesiser operation. (d) The UV signal from the injection of dye into the feed tank at 0.6 kg min^{-1} , in recirculating synthesiser operation, for fast dye injection, and (e) with controlled dye injection at a constant rate over one residence time.

Due to the high degree of PFR behaviour of the system, the reagents for DSP synthesis must be added carefully. The effect of uncontrolled reagent addition was simulated by using fast injections of dye into the feed tank of the synthesiser, which was operated in recirculation mode. This experiment simulated the fast addition of reactants for the chain extension and deprotection reactions. The UV signal of the fast dye injections is shown in **Fig. 7.4d**. When the dye was injected quickly, the dye remained as a highly concentrated plug that travelled around the system with slow mixing and slow dispersion. This highly concentrated plug took over six residence times until the concentration was constant in the

synthesiser. Not only does this result in slower reactions in the low concentration zones, but the four times higher initial concentration in the synthesiser leads to the formation of impurities. For oligonucleotides, high reactant concentrations can lead to $n+1$ and depurination impurities [332].

Therefore, the controlled addition of reagents was investigated. The same concentration of dye was added at a constant and controlled rate, over one system residence time, shown in **Fig. 7.4e**. Adding the reagents over a single residence time maintained a consistent concentration in the synthesisers, with the maximum concentration < 1.2 times the steady state concentration. This maximises the average rate of reaction in the synthesiser and minimises the formation of $n+1$ or depurination impurities. Therefore, for the DSP synthesis, all reagents were added at a constant rate over one system residence time.

7.3.3 Hub Optimisation

After construction of the synthesiser, a soluble hub must be chosen. The hub is attached to the 3' end of the oligonucleotide and is used to increase the size of the growing oligonucleotide, therefore ensuring $\sim 100\%$ membrane rejection. Additionally, the hub affects the DSP solubility. For oligonucleotides, this is particularly significant because the growing oligonucleotide has low solubility in the chain extension solvent, acetonitrile. Previous studies have shown that reacting an oligonucleotide to a PEG chain significantly increases the solubility of the oligonucleotide in acetonitrile [28,112–114]. This technique was previously used by Bonora et al. to synthesise a 20-mer oligonucleotide on a PEG 12 kDa hub using liquid phase reactions and purification with precipitation [113]. This was achieved due to the improved solubility of the oligonucleotide after conjugation to PEG. Therefore, PEG was investigated as a soluble hub for the membrane enabled synthesis of pharmaceutical length oligonucleotides.

Optimisation of the hub requires selection of a PEG molecular weight to attach to the oligonucleotide. A high molecular weight hub is predicted to increase the oligonucleotide rejection and increase the oligonucleotide solubility. However, a high molecular weight hub also increases the solution viscosity and decreases the rate of the reactions. To efficiently permeate the tritylated building blocks, which vary between 700 and 900 Da, a common rule of thumb is to select a membrane with a MWCO approximately six times higher than the molecular weight of the permeating species, corresponding to

a MWCO ~ 5 kDa [134]. Therefore, for DSP synthesis, where the hub must have $\sim 100\%$ rejection, the hub molecular weight must be significantly higher than 5 kDa.

An additional important factor for oligonucleotide synthesis is a requirement to perform the reactions and the separation at a similar concentration, which is due to the use of a single apparatus one-pot synthesiser. For the oligonucleotide synthesis, the reactions must be performed at ~ 10 mM. Therefore, the membrane separations must also be performed at ~ 10 mM. At these concentration, mass transfer effects are very important [339,340]. However, there has been little research on the effect of mass transfer on constant volume diafiltration in organic solvents.

Therefore, a preliminary investigation into the optimal hub was completed. This work used three different linear molecular weight PEGs, 10 kDa, 20 kDa, and 35 kDa, and examined the effect of mass transfer on the separation performance of these potential hubs, shown in **Fig. 7.5**. Each potential hub was individually added to the synthesiser, which contained acetonitrile and a molecule of similar molecular weight and concentration to the building block, sucrose octaacetate (SoA). The concentration of the hub was systematically increased from 0 g L^{-1} to 50 g L^{-1} . The rejection of the two solutes (PEG and SoA), and the permeance, was measured throughout.

The effect of PEG concentration on the membrane flux is shown in **Fig. 7.5a-c** for 10kDa, 20kDa, and 35 kDa PEG respectively. Increasing the PEG concentration caused the flux to decrease significantly. For 35 kDa PEG, the flux declined from 59 mL min^{-1} at 0 g L^{-1} PEG to 8 mL min^{-1} at 45 g L^{-1} PEG at 20 Bar. This flux decline is due to concentration polarisation. Concentration polarisation is often significant in concentrated systems and is a major limitation of membrane separations [341–345]. Increasing the PEG concentration in the bulk significantly increases the PEG concentration at the membrane surface, which causes the flux to decrease. The lower flux can be attributed to a reduction of the membrane hydraulic permeability due to the presence of a gel layer which effectively increases the membrane resistance, or attributed to an increase in the osmotic pressure which reduces the driving force across the membrane. Due to the high osmotic pressure of high molecular weight PEG at high

concentrations, it is predicted that the decreased flux at high PEG concentrations is due to a combination of both phenomena [346].

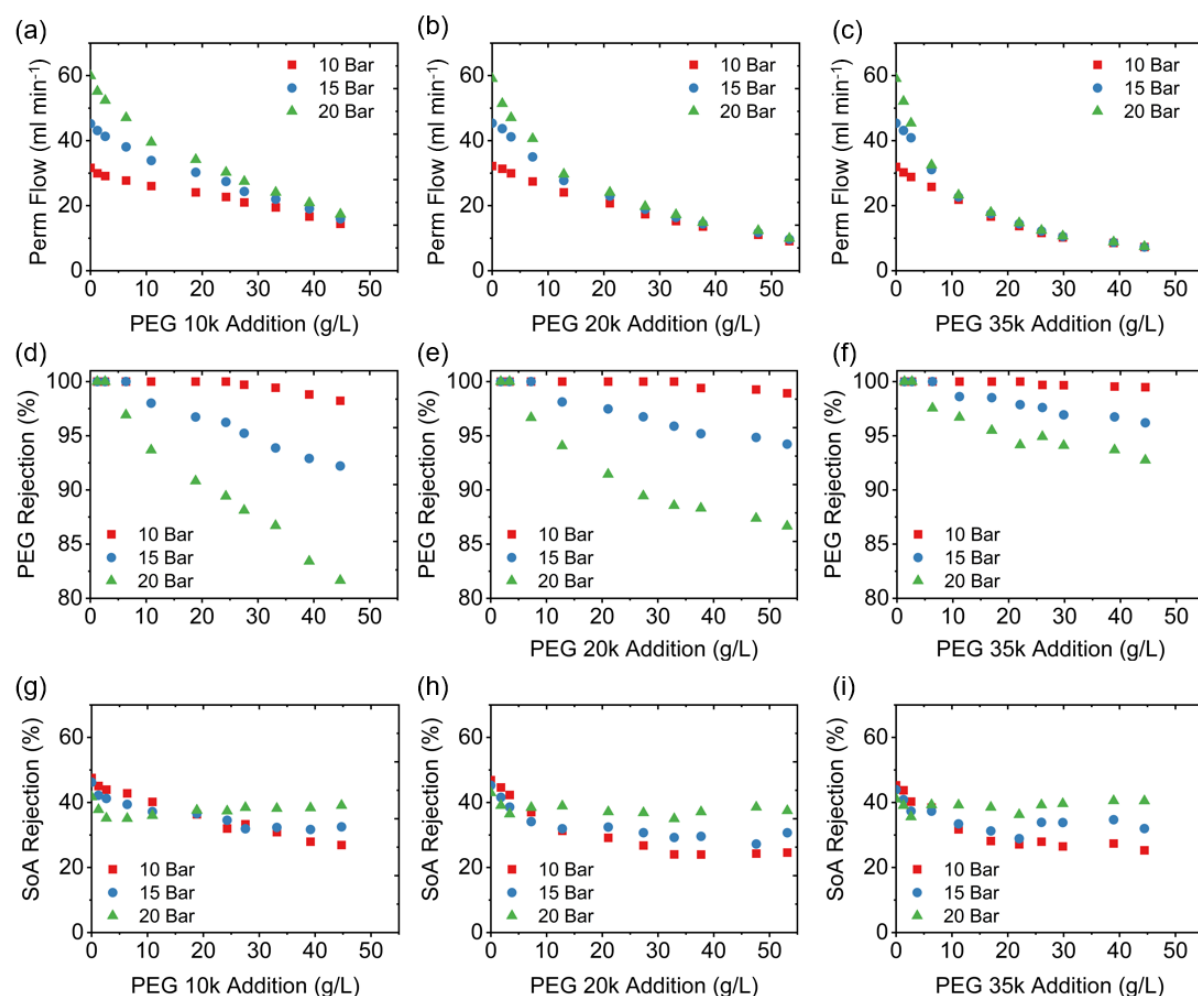


Fig. 7.5 The performance of M-97.5 membranes on addition of PEG to a system of 10 g L^{-1} SoA in acetonitrile. The rig was operated at 2 kg min^{-1} cross-flow rate, 30°C , and 10, 15, and 20 bar. The permeate flow rate on addition of (a) PEG 10 kDa, (b) PEG 20 kDa, (c) PEG 35 kDa. The change in PEG rejection on addition of (d) PEG 10 kDa, (e) PEG 20 kDa, (f) PEG 35 kDa. The change in SoA rejection on addition of (g) PEG 10 kDa, (h) PEG 20 kDa, (i) PEG 35 kDa.

The extent of flux decline was highly dependent on the molecular weight of the PEG and the system pressure. The membrane flux with a high PEG molecular weight was significantly lower than the flux of the system with a lower PEG molecular weight. This is because the addition of high molecular weight polymers leads to higher solution viscosities and slower diffusion, resulting in increased concentration

polarisation and a higher PEG concentration at the membrane surface. Additionally, high molecular weight polymers exert higher osmotic pressure at the same concentration, which further reduces the flux [347–349]. Therefore, to maximise the system flux, low molecular weight hubs are favourable for oligonucleotide synthesis.

Additionally, the flux decline due to concentration polarisation was highly dependent on the system pressure. Increasing the system pressure resulted in a significant increase in the concentration of PEG in the boundary layer. This increased the osmotic pressure and compressed the membrane gel layer, which resulted in only a small increase in the flux when the pressure was increased [350,351]. For this system, at 45 g L⁻¹ PEG 35 kDa, the flux was 7.30 mL min⁻¹, 7.38 mL min⁻¹, and 7.41 mL min⁻¹ at 10 bar, 15 bar, and 20 bar respectively. Therefore, at these conditions, increasing the pressure has a negligible benefit on the rate of filtration.

The effect of increasing the PEG concentration on the PEG rejection is shown in **Fig. 7.5d-f** for 10 kDa, 20 kDa, and 35 kDa PEG respectively. For a successful oligonucleotide synthesis, the hub must have a high rejection. Using the PBI membrane, the PEG was fully rejected by the membrane (>99.5 % rejection) for all three molecular weights at low concentrations < 2 g L⁻¹. However, as the concentration of PEG in solution was increased, and as the pressure increased, the PEG rejection decreased. This was attributed to the increased concentration on the membrane surface due to concentration polarisation which had several contributing effects to decrease the PEG rejection.

The first factor is because a high PEG concentration on the surface of the membrane causes a significant difference between the observed and real rejection, which results in a lower observed rejection at higher concentrations [343,350]. The second reason is because an increased PEG concentration on the membrane surface increases the concentration gradient across the membrane. This increases the PEG driving force across the membrane and decreases the PEG observed rejection [343]. The third reason is attributed to the behaviour of polymers in semi-concentrated solutions. It has been previously shown that the shear flow at the entrance of a membrane pore can distort the shape of linear polymers into elongated structures due to a high degree of molecular flexibility in polymers [352]. This enables the

polymer to pass through pores smaller than the hydrodynamic diameter of the polymer. As the concentration of PEG in the bulk increases, the concentration at the membrane surface increases and the 'critical flux', defined as the flux which enables sufficient shear deformation for the polymer to change shape in solution and pass through the pore, decreases. This increases the concentration of PEG passing through the membrane due to the high molecular flexibility and shear deformed permeation [233,353,354]. These reasons all contribute to the significantly lower observed rejection at high PEG concentrations. Therefore, to achieve a high hub rejection, the concentration polarisation must be reduced. This can be achieved by minimising the system pressure, as an increased pressure resulted in a significant decrease of the PEG rejection due to the increased concentration polarisation at high system pressures. Additionally, increasing the local turbulence and increasing the system temperature can reduce concentration polarisation.

The decrease in rejection with increased PEG concentration was less significant for a higher molecular weight PEG. Increasing the PEG molecular weight results in an increased concentration of the PEG at the surface of the membrane due to a higher viscosity and slower diffusion. This can result in a lower rejection. However, increasing the PEG molecular weight also increases the PEG hydrodynamic diameter. This can result in less permeation through the membrane and a higher observed rejection [233]. For this system, the effect of the larger hydrodynamic diameter offset the effect of the higher concentration at the membrane surface and the higher molecular weight PEG had higher rejection. Therefore, although the higher molecular weight PEG hub had a significantly lower flux, the higher rejection makes the high molecular weight hub more economical for oligonucleotide synthesis.

The SoA rejection was also measured and found to be a function of the PEG concentration, shown in **Fig. 7.5g-i** for 10 kDa, 20 kDa, and 35 kDa PEG respectively. SoA has a similar molecular weight to the building block and the effect of increasing the PEG concentration is expected to have a similar effect on SoA and on the building block [125].

The SoA rejection at 0 g L⁻¹ PEG was between 42 and 47%, and the rejection decreased as the pressure increased. This was attributed to a small amount of SoA concentration polarisation at high pressure,

which increased the SoA concentration on the surface of the membrane and led to a decrease in the observed rejection.

However, as the concentration of PEG in the system was increased, the rejection of the SoA changed. This was attributed to a switch from membrane-controlled rejection at low PEG concentration, to a combination of membrane-controlled rejection and PEG boundary layer-controlled rejection at high PEG concentration. The PEG boundary layer is predicted to have multiple effects on the rejection of a permeating species. The PEG layer acts as an additional resistance layer, forming a 'second dynamic membrane' and increasing the rejection of the SoA [355,356]. However, there is a competing effect of slower SoA diffusion in the PEG boundary layer which increases the concentration polarisation of the SoA. This increases the concentration of SoA at the membrane surface and results in a decrease in the observed rejection.

Depending on the pressure and PEG concentration, the rejection of the SoA did change. However, the rejection of SoA was maintained between 25% and 50%, with the highest rejection observed at the lowest PEG concentration. With this range of rejections, the SoA can be removed using diafiltration at all tested conditions. For 25% rejection, six system volumes would be needed to wash out 99% of the building block, compared to nine system volume for 50% rejection. Therefore, although the SoA rejection is system dependant, these changes are not critical to the success or economics of membrane enabled DSP synthesis.

The results of this study show that a high PEG concentration has a negative effect on the PEG rejection. The rejection can be increased by minimising concentration polarisation and increasing the molecular weight of the PEG hub. However, at high PEG concentrations, the PEG rejection was <100% for all tested PEG molecular weights and pressures. Therefore, it is infeasible to use these PEG molecules as hubs for the synthesis of long oligonucleotides, and the concentration of PEG must be decreased for this system to be feasible.

One method of reducing the concentration of the PEG hub in the synthesiser is to use a multi-arm hub. Using a 4-arm, or 8-arm, 10 kDa hub reduces the required system concentration of PEG from 100 g L⁻¹

¹ for a linear PEG to 25 g L⁻¹ or 12.5 g L⁻¹ respectively, while maintaining a constant oligonucleotide concentration, of 10 mM. This is achieved while maintaining a hydrodynamic diameter which should be large enough to have a high hub rejection. To investigate using multi-arm hubs, three hubs were tested. These were linear 10 kDa, 4-arm 10 kDa, and 8-arm 10 kDa hub, and the structure of these is shown in **Fig. 7.6a-c** respectively. The multi-arm PEGs have a central flexible core to link all of the arms, and all of the arms are approximately the same molecular weight.

To investigate the use of multi-arm hubs, the effect of the hubs on membrane rejection was tested at a range of operating conditions. Concentration polarisation is known to be more significant at high pressure and low cross-flow rate. Therefore, to prove that the decreasing rejection was due to concentration polarisation, a central composite design (CCD) optimisation of pressure and cross-flow rate was used to compare different operating conditions on the flux and rejection of linear 10 kDa, 4-arm 10 kDa and 8-arm 10 kDa hubs at a constant concentration, 25 g L⁻¹.

The sampling points taken for this CCD study are shown in **Fig. 7.6d**. A parameter space from 0.75 kg min⁻¹ to 2 kg min⁻¹ cross-flow rate and from 2 bar to 12 bar transmembrane pressure was investigated. Eight data points were analysed singly, and five data points were analysed doubly to calculate the error of this technique. The standard deviation on all permeance measurements was < 0.7 LMHBar and the error on all rejection measurements was < 0.25 %, shown in **Fig. 7.6e**.

The permeance of the linear 10 kDa, 4-arm 10 kDa and 8-arm 10 kDa hubs is shown in **Fig. 7.6f-h** respectively. For all hubs the highest permeance was between 40 LMHBar and 45 LMHBar, corresponding to 2 kg min⁻¹ and 2 bar. However, increasing the pressure, or decreasing the cross-flow rate, decreased the permeance. This is the characteristic effect of concentration polarisation and shows that the changes in permeance are due to mass transfer limitations and concentration polarisation [345,350]. It is noteworthy that the trends and the permeances were relatively consistent between the three different potential hubs. This showed that the extent of concentration polarisation was mostly dependent on the hydrodynamic conditions, and the permeance was relatively independent of the number of arms on the hub.

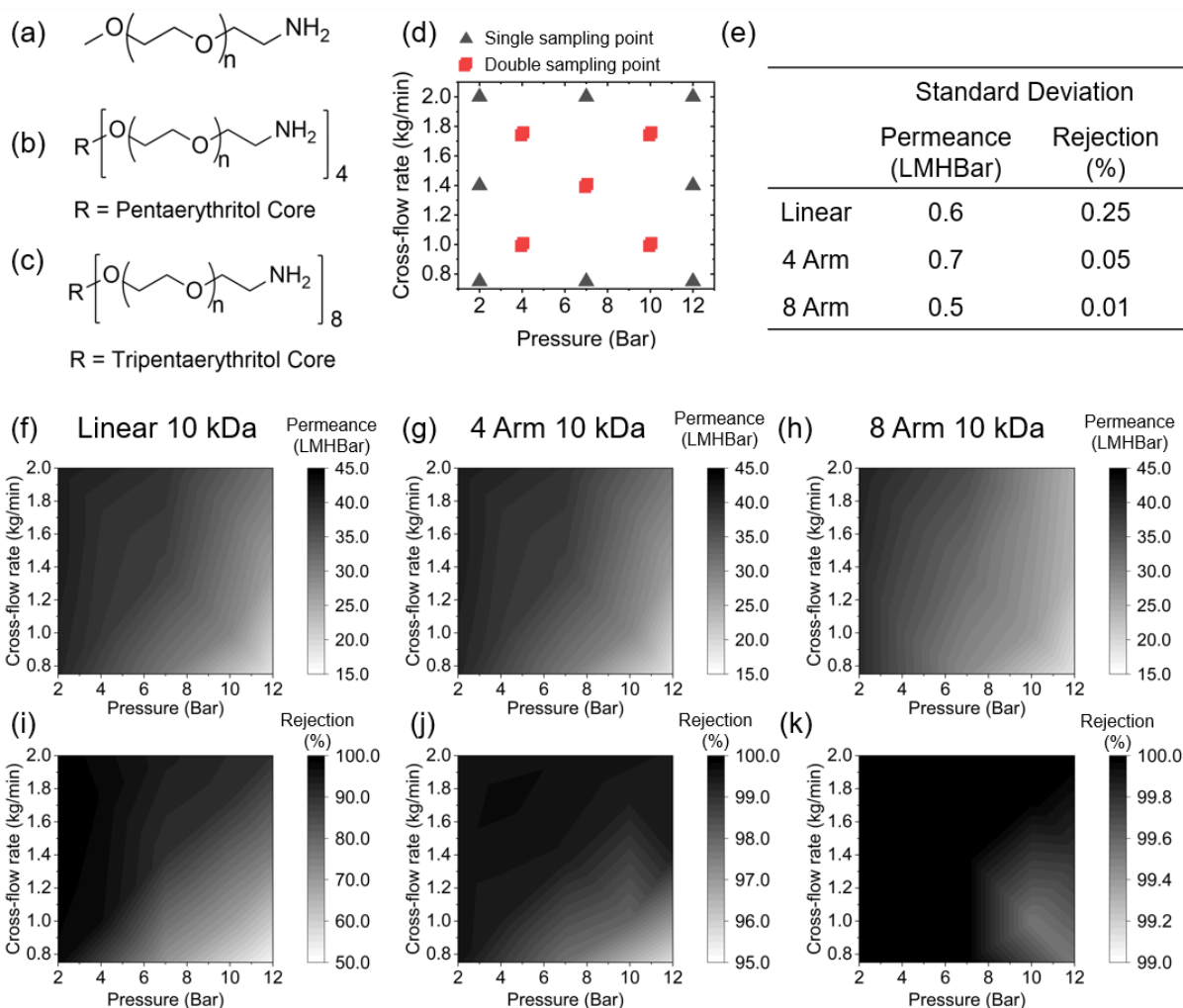


Fig. 7.6 The flux and rejection change of M-97.5 membranes with 25 g L^{-1} 10 kDa PEG with various arm numbers at different cross-flow rates and pressures. The structure of (a) linear PEG which had a total $M_w = 10 \text{ kDa}$, (b) 4-arm PEG which had a total $M_w = 10 \text{ kDa}$, (c) 8-arm PEG which had a total $M_w = 10 \text{ kDa}$. (d) The CCD data points which were investigated in the parameter space from 2 bar to 12 bar, and 0.75 kg min^{-1} to 2 kg min^{-1} . The black triangle data points were taken once, and the double red squares were taken as two repeats. (e) The standard deviation from the CCD study. The change of permeance with changing operating conditions for (f) linear PEG 10 kDa, (g) 4-arm PEG 10 kDa, (h) 8-arm PEG 10 kDa. The change of PEG rejection with changing operating conditions for (i) linear PEG 10 kDa, (j) 4-arm PEG 10 kDa, (k) 8-arm PEG 10 kDa.

The rejection of the linear 10 kDa, 4-arm 10 kDa and 8-arm 10 kDa hubs is shown in **Fig. 7.6i-k** respectively. For all hubs, the rejection with a low extent of concentration polarisation was $\sim 100\%$.

However, as the extent of concentration polarisation increased, the rejection decreased, with the lowest rejection corresponding to the highest pressure and lowest cross-flow rate, 12 bar and 0.75 kg min⁻¹. This confirmed the hypothesis that the lower rejection was due to concentration polarisation. In this CCD investigation, the rejection was significantly dependent on the number of arms, and an increased number of arms corresponded to a higher rejection at the system conditions where concentration polarisation was significant. For the lowest rejection in the CCD parameter space, the rejection was 51.9%, 95.7% and 99.5% for the linear 10 kDa, 4-arm 10 kDa, and 8-arm 10 kDa hubs respectively.

The improved rejection of multi-arm hubs is attributed to a change in the 'critical flux'. Increasing the number of arms decreases the hydrodynamic diameter of the polymer and should result in a decrease of the rejection. However, the effect of the size difference is offset by a change of the critical flux. The critical flux is the flow rate through a pore which is required to elongate the polymer chain into a shape which makes it easier for the polymer to permeate through the pores. This critical flux decreases with increasing polymer concentration at the membrane surface. However, it has been shown that the critical flux is significantly higher in multi-arm polymers and increases with increased branching. Therefore, by using a higher number of arms, a high rejection can be maintained at high concentrations, even at low cross-flow rates and high pressure [353,357,358].

From these investigations, it was shown that a multi-arm hub was optimum for a high rejection in mass transfer-controlled membrane separations. Therefore, a preliminary oligonucleotide synthesis was attempted using a 4-arm 10 kDa PEG hub at 25 g L⁻¹. However, the growing PEG-oligonucleotide became increasingly insoluble as the oligonucleotide chain length increased. This led to the oligonucleotide crashing out of solution before the full-length 20-mer product was manufactured. Although PEG was hypothesised to increase the solubility of the oligonucleotide, this unsuccessful test showed that there was a minimum molecular weight of PEG per oligonucleotide chain which is required to solubilise the oligonucleotide.

Bonora et al. showed that a 5 kDa PEG could solubilise an 8-mer oligonucleotide. However, the PEG molecular weight needed to be increased to 12 kDa to solubilise a 20-mer oligonucleotide [113]. Based

on this data, a higher PEG molecular weight improves the solubility of the oligonucleotide, and approximately 10kDa of PEG is needed to solubilise each 20-mer oligonucleotide. Therefore, there are a number of potential interesting hub choices including monofunctional linear 10 kDa PEG, difunctional 20 kDa PEG, or 4-arm 40 kDa PEG (large quantities of 8-arm 80 kDa PEG were not commercially available). However, due to the requirement for the hub to be multi-armed to achieve a high membrane rejection, the 4-arm 40 kDa PEG hub was the only viable candidate for oligonucleotide DSP synthesis. The new reaction scheme using 4-arm 40 kDa PEG as a hub is shown in **Fig. 7.7**. The hub was linked to the oligonucleotide with a cleavable sarcosine-succinate linker to ensure that the oligonucleotide could be cleaved from the hub following the synthesis of the 20-mer.

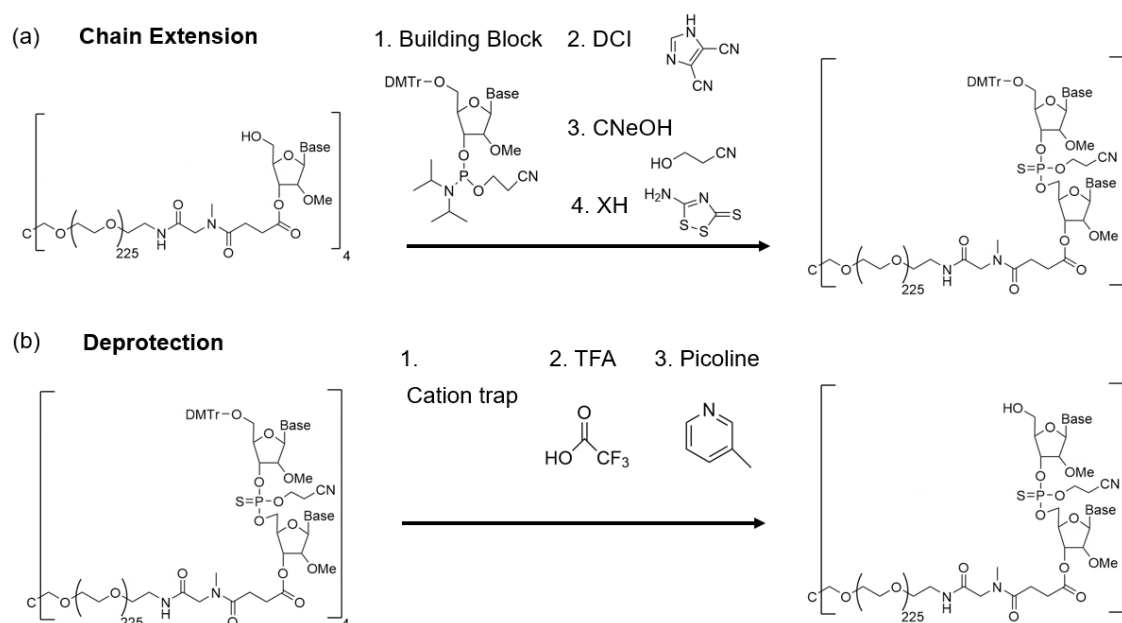


Fig. 7.7 (a) The first oligonucleotide chain extension on a 4 Arm-PEG 40 kDa-sarcosine-succinate hub, and (b) the following deprotection reaction for oligonucleotide synthesis. Each arm corresponds to an average of 10 kDa PEG or 225 PEG units per arm.

7.3.4 Membrane Screening

Using this hub, a membrane was required which had ~100% rejection of the 4 Arm-PEG 40 kDa-sarcosine-succinate-mU-DMTr starting material at the required synthesiser starting concentration, 100 g L⁻¹ in acetonitrile. Four PBI membranes were selected for screening, which all had 100% rejection of

linear 35 kDa PEG in a dilute system. These were: 14 wt% PBI modified with 2-MEA and 2kPPG (14_2-MEA and 14_2kPPG respectively), and 18 wt% PBI modified with 2-MEA and 2kPPG (18_2-MEA and 18_2kPPG respectively). The hub rejection for each membrane is shown in **Fig 7.8a** and the corresponding permeance in **Fig 7.8b**.

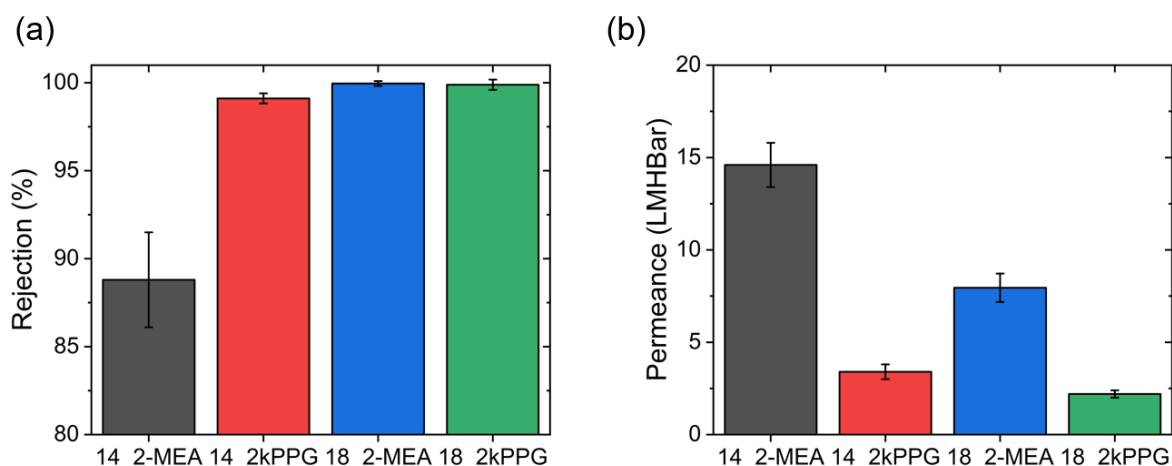


Fig. 7.8 (a) The rejection of the hub, 4 Arm-PEG 40 kDa-sarcosine-succinate-mU-DMTr, at 100 g L^{-1} in acetonitrile, 2 kg min^{-1} cross-flow, 30°C , and 10 bar. The membranes were from 14% (14_) and 18% (18_) casts which were modified with 2-MEA or 2kPPG. (b) The permeance of the membranes at the same conditions.

The rejection of the 14_2-MEA membrane was lowest, 88.8%. This was significantly lower than the rejection of a linear 35 kDa PEG in a dilute system, which was $\sim 100\%$. The lower rejection in the real system is attributed to significant concentration polarisation and, because of the low rejection, this membrane cannot be used in the oligonucleotide synthesis. The rejection of the other three tested membranes was over 99%. However, the hub rejection of the 14_2kPPG was significantly lower than the hub rejection of the 18 wt% modified membranes, 99.1% compared to $>99.8\%$. An average hub rejection of 99.1% throughout a 20-mer synthesis results in a final oligonucleotide yield of $\sim 16.5\%$. Therefore, the 14_2kPPG membrane was also eliminated as a membrane choice for oligonucleotide synthesis. The rejection of the two 18 wt% membranes was similar, 99.89% and 99.96% for 18_2-MEA and 18_2kPPG respectively. However, the permeance of the 18_2-MEA membrane was significantly

higher, 7.95 LMHBar and 2.2 LMHBar for 18_2-MEA and 18_2kPPG respectively. Therefore, to achieve faster diafiltrations, the 18 wt% high molecular weight PBI modified with 2-MEA (18_2-MEA) was selected as the optimum membrane for oligonucleotide synthesis.

7.3.5 Oligonucleotide Synthesis

A 20-mer oligonucleotide, M23D (+2-18), was synthesised iteratively using the strategy in **Section 7.3.1**. The synthesiser designed in **Section 7.3.2** was used, with the hub optimised in **Section 7.3.3** and the membranes optimised in **Section 7.3.4**.

The successful synthesis of an oligonucleotide, using the platform technology of membrane enabled liquid phase synthesis, requires effective membrane separations to be successful. A high purity oligonucleotide can only be achieved by fully purifying the oligonucleotide in every cycle and a high yield synthesis can only be achieved by maintaining a high hub rejection throughout the synthesis. Therefore, in order to continuously verify the membrane performance, the separation efficiency was measured at every step of the synthesis. This is possible due to the entirely liquid phase nature of this process, which is unique to membrane enabled liquid phase synthesis.

An example of separation efficiency monitoring is shown in **Fig. 7.9a**, corresponding to the DF1 and DF2 of the 3-mer coupling of a 'C' building block. The hub rejection was measured throughout the DF1 and DF2, shown in **Fig. 7.9a**. The hub rejection was relatively constant throughout the diafiltration, with a high average rejection of 99.9%. This verified that the membrane screening was accurate, and the presence of the reaction debris in the system do not significantly affect the hub rejection. If the hub rejection is maintained at 99.9% throughout the synthesis of the 20-mer, a high final yield of ~82% should be achieved.

For the separation monitoring, the removal of the building block was also investigated. The building block is the largest permeating species in the system, and if any building block remains it will lead to significant impurity formation. In DF1, four system volumes were permeated through the membrane. The rejection of the building block was between 30% and 40% throughout DF1, and the building block concentration was decreased to ~17% of the initial concentration. In DF2, after detritylation, the

building block rejection decreased to ~20%. The lower rejection in DF2 was due to the smaller size and increased hydrophilicity of the building block after the trityl protecting group was removed. After DF2, <1% of the building block remained. This proved that the membranes were effective at fully removing the unreacted building block, even with the additional transfer resistance due to the concentrated hub boundary layer. Because this separation was highly effective, there should be no contamination of building block in the next chain extension cycle and the PBI membranes should synthesise a high purity oligonucleotide.

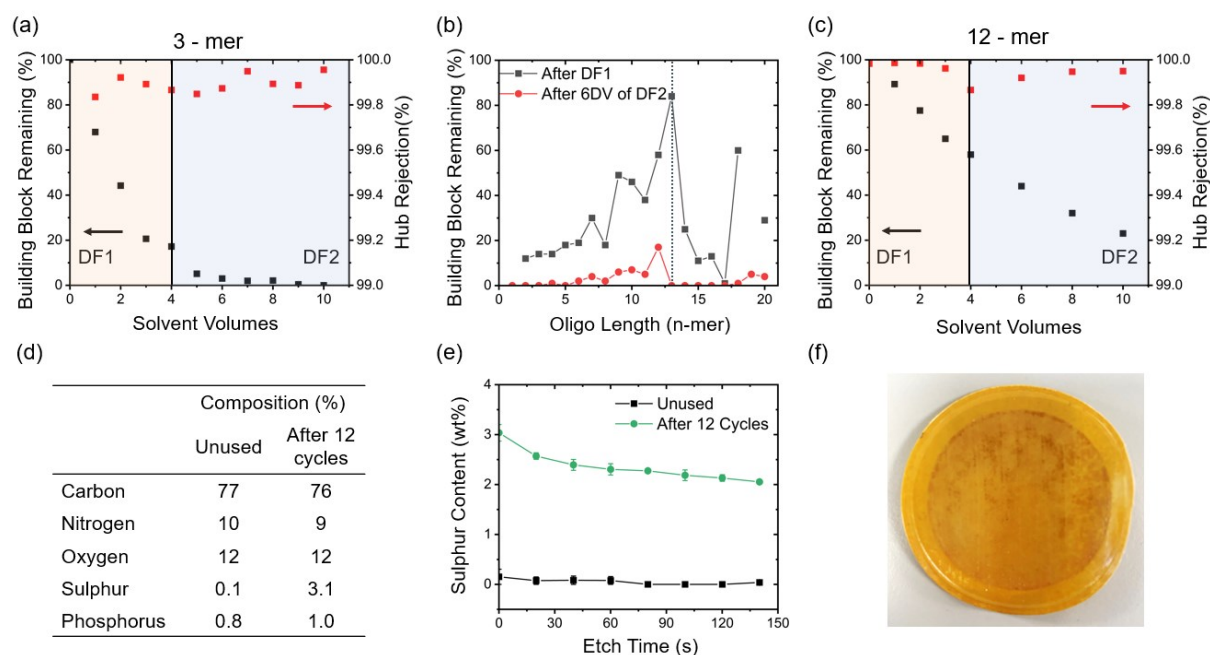


Fig. 7.9 (a) The building block remaining and hub rejection, monitored in DF1 and DF2 of the 3-mer. (b) The building block remaining after DF1, and six system volumes (DV) permeated in DF2 for each oligonucleotide length. Here, the building block could not be monitored at the end of DF1 of the 1-mer and 19-mer stages due to overlap with other components on the UHPLC. The vertical dashed line represents the change to fresh membranes. (c) The building block remaining and hub rejection, monitored in DF1 and DF2 of the 12-mer. (d) The atomic composition of the unused and used membranes. (e) The sulphur content of the unused and used membranes. (f) The fouled membrane after filtration of the cation trap in acetonitrile.

However, it was observed that the membrane performance declined throughout the 20-mer synthesis. Throughout the synthesis, the building block remaining after DF1 and after six system volumes of DF2 was constantly monitored, shown in **Fig. 7.9b**. Throughout the first 12 chain extensions, the remaining average building block increased as the oligonucleotide length increased.

The increasing building block remaining was analysed in detail by investigating the building block wash out during the 12-mer chain extension, shown in **Fig. 7.9c**. The 12-mer chain extension used the same 'C' building block as the trimer coupling, so the size of the permeating molecule was identical. However, for the 12-mer, 58% of the building block remained after DF1, compared to 17% at trimer. Additionally, after six system volumes of DF2, 18% building block still remained, compared to <1% at trimer. Due to the high building block rejection, the 12-mer DF2 required an additional five system volumes to reduce the remaining building block to ~1% of the initial unreacted building block, in order to prevent impurities forming in the next chain extension.

This change of membrane performance could be due to changes to the membrane, or it could be due to changes to the system behaviour because of the presence of a growing mass of oligonucleotide. To test this, and because of the slow removal of the building block, the membranes were replaced with fresh membranes after DF1 of the 13-mer.

After replacement of the membranes, the performance of the new membranes was tested by monitoring the building block washout. The fresh membranes removed the building block at a similar rate to the initial membranes at the start of the process, and <1% building block remained after six system volumes of DF2 from 13-mer to 17-mer, shown in **Fig. 7.9b**. This showed that there is a high probability that it was the membranes which were changing during the first 12 chain extension cycles and the performance decline was not due to the presence of the growing oligonucleotide in the synthesiser. To further verify this, the membranes which were removed from the synthesiser were washed and the pure solvent permeance was measured. After 12 full synthesis cycles, the pure solvent permeance was 45% lower than the fresh membranes, confirming that the membrane performance had declined.

The changing performance of the membranes was hypothesised to be due to fouling. The membranes were visually examined after removal from the synthesiser and a thin surface coating of foulant particles was visible on the top surface of the membrane. To understand this fouling, the elemental composition of the membranes was analysed by XPS. After 12 full chain extension cycles, the atomic composition of the carbon, nitrogen, and oxygen was virtually unchanged, shown in **Fig 7.9d**. Additionally, there was very little change of the phosphorus content, which showed that the oligonucleotide was not significantly reacting to or adsorbing to the membrane. However, after 12 full chain extension cycles, the sulphur content of the membrane was significantly increased, from 0.1 ± 0.2 wt% for the fresh membrane to 3.1 ± 0.2 wt%. Therefore, the foulant which was visible on the surface of the membrane was attributed to sulphur containing particles. The sulphur concentration was also investigated at increasing depth through the membrane, shown in **Fig. 7.9e**. The sulphur content in the membrane declined with increasing depth into the membrane, which is characteristic of membrane fouling with a sulphur-based compound.

Due to the foulant being sulphur-based, the fouling was hypothesised to be due to the addition of small quantities of insoluble solid particles in the thiol-based cation trap. The manufacture of the cation trap used solid sulphur containing particles, and the addition of these particles into the synthesiser would lead to pore blocking, resulting in a decline of the permeance and selectivity of the membrane.

To prove this, the pure acetonitrile permeance of a membrane was compared before and after filtering a solution of the thiol-based cation trap. After filtration, the permeance decreased significantly, by ~21% relative to the filtration of the pure solvent, and there was a thin coating of particles visible on the membrane surface, shown in **Fig. 7.9f**. This verified that the foulant was at least partly due to the thiol-based cation trap, which caused the decreasing membrane performance.

One of the critical differences between this process and all other membrane applications is the high value of the product which is continuously manufactured on the retentate side. This product must have ~100% retention inside the synthesiser and the process cannot use any cleaning steps or purge steps. Therefore, the foulant must be prevented from entering the synthesiser, as there are no methods to

remove it once it is inside the synthesiser without also losing a significant quantity of the hub. To stop the foulant entering the synthesiser, the thiol-based cation trap must be prefiltered using a sacrificial nanofiltration membrane in all future syntheses. This membrane must be tighter than the membrane used in the synthesiser and will remove the foulant prior to the foulant entering the synthesiser.

Additionally, anti-fouling membranes can be used to ensure long term membrane performance in DSP synthesis. The performance decline of the membranes during the oligonucleotide synthesis showed the importance of removing every source of foulant. However, this is incredibly difficult to achieve and some foulant will always enter the synthesiser. Therefore, an important future improvement is to use the 2kPPG membranes, designed in **Chapter 6**, which showed good anti-fouling performance in acetonitrile. This will enable the impact of the foulant to be reduced and the lifetime of the membranes should be increased.

Despite the decreasing selectivity and the significant effect of fouling, the building block was removed, and the hub rejection was high at every chain extension. This proved that these membranes were a significant improvement compared to the membranes used in previous membrane enabled oligonucleotide synthesis and shows the viability of membrane enabled liquid phase synthesis to synthesise pharmaceutical length oligonucleotides [7,29].

After 20 iterations, the hub-oligonucleotide was removed from the synthesiser. A dry mass of 32.2 g was recovered. This corresponded to 50% yield, which is comparable to the yield from SPS [4]. The yield from this process was significantly better than the previous studies of membrane enabled defined sequence polymer synthesis, which had a maximum yield of 39% for a 9-mer oligonucleotide [7,29]. The high final yield showed that a high hub rejection was maintained throughout the entire synthesis, greater than 99.6%. This shows that the hub and membrane optimisation experiments were effective, and a hub-membrane combination was identified which can successfully synthesise oligonucleotides. Additionally, the high yield, after over 200 system volumes of permeation, shows the improved performance of the PBI membranes which were developed in **Chapter 4-6** of this work.

However, there was still 50% yield loss in the 20-mer synthesis. This was attributed to three main reasons, and it is expected that the yield from this process can be increased in future syntheses with a small number of improvements.

Some of this yield loss was due to permeation through the membranes. Based on the average hub rejection of ~99.9%, approximately 18% of the oligonucleotide was lost due to permeation through the membrane. This shows the significant membrane development which has been made in this work and shows that it is now possible to use membrane enabled defined sequence oligonucleotide synthesis to manufacture pharmaceutical length oligonucleotides. However, 18% yield loss is still significant, and it may be possible to reduce this yield loss by making small changes to the membranes or small changes to the hub. These are noteworthy topics of future work and are discussed in **Chapter 8**.

A significant amount of the yield was also lost due to comprehensive sampling at all stages of the synthesis. With increased process understanding, reduced sampling, and increased scale, the final yield will be higher. However, for this proof-of-concept study, comprehensive sampling was essential to understand the advantages and limitations of this process.

Finally, it is expected that a significant amount of the yield was lost during the change of the membranes after 13-mer DF1. By reducing the amount of foulant which is added to the synthesiser and using the anti-fouling membranes developed in **Chapter 6**, the membranes should maintain consistent performance over a full synthesis. Therefore, this yield loss can be prevented in future syntheses.

After measuring the yield, the final oligonucleotide was cleaved from the hub and globally deprotected. The final oligonucleotide was analysed for UV purity and MS purity with UHPLC by Exactmer Ltd. The scope of this work predominantly focused on optimising the membranes, hub, and synthesiser design, with the oligonucleotide synthesis chemistry designed and optimised by Exactmer Ltd. Therefore, the impurity analysis in this work focussed on the impurities which are formed due to the choice of membranes, hub, or synthesiser design.

The UHPLC UV signal is shown in **Fig. 7.10a** and the critical peaks are summarised in **Fig. 7.10b**. The chromatogram has one single main peak, with one small UV active peak at the very start of the method,

and two small peaks at the end of the method. The main peak corresponded to the full-length oligonucleotide, along with the close eluting impurities. By UV the crude purity of the globally deprotected oligonucleotide was 97.5%.

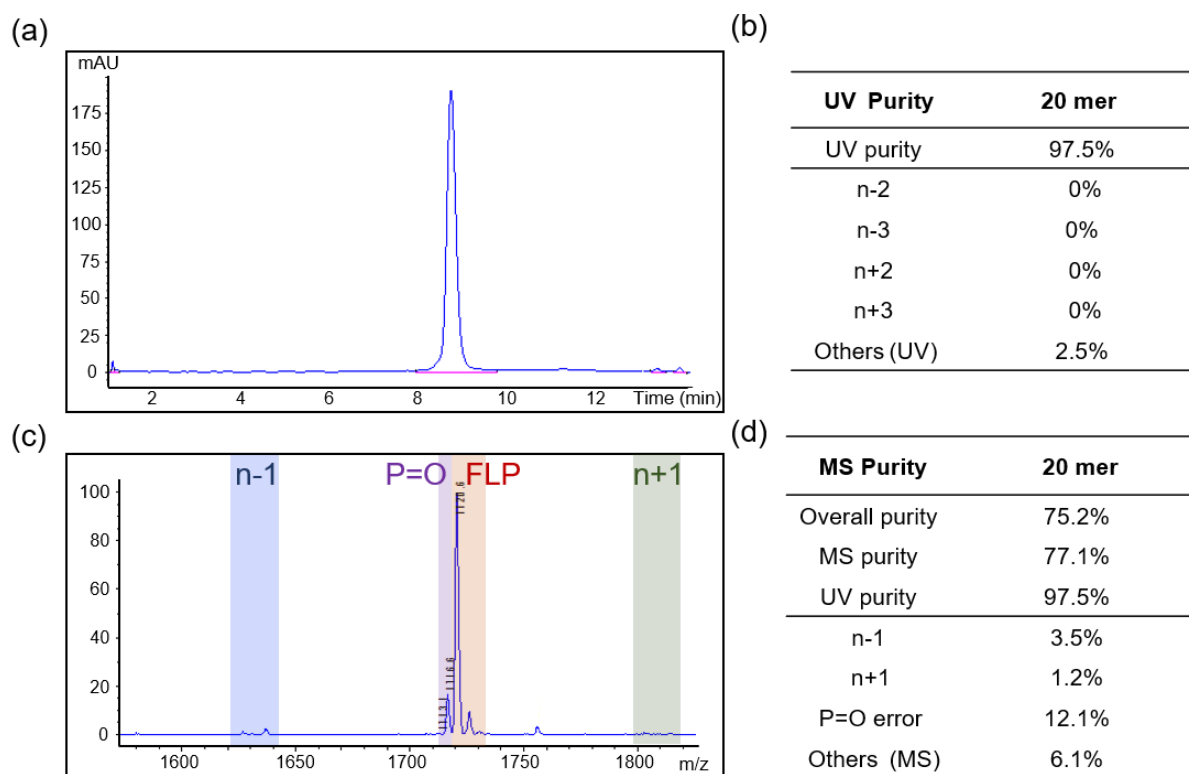


Fig. 7.10 (a) UV signal from ion pair-reversed phase chromatography. (b) Summary table of identified UV impurities. (c) MS signal of the main UV peak from ion pair-reversed phase chromatography, focused on the (-4) charged species. The n-1, P=O, full length product (FLP), and n+1 impurity regions are highlighted. (d) Summary table of known MS impurities.

The UHPLC method was designed to identify the presence of oligonucleotide sequences which are significantly shorter than the full-length product (n-2, n-3...etc) [359]. It is noteworthy that this does not account for the presence of n-1 impurities. The n-1 sequences cannot be separated using this UHPLC method and they will be discussed later in this section. If the building block was not fully removed at every chain extension, the remaining building block would be chain extended in every subsequent reaction to form a shortmer sequence (a chain of oligonucleotide bases with a lower number of monomers than the full-length product) [360]. By UV, the final product contained no shortmers. This shows that the building block was fully removed at every chain extension and proves that the PBI

membranes are a highly effective purification method for oligonucleotide synthesis. The effectiveness of the purifications using PBI membranes resulted in a higher UV purity from membrane enabled liquid phase synthesis than the UV purity from industrial SPS [4,24,361,362].

However, by UV, three impurity peaks were observed, which were not the main product. The other three UV active peaks are impurities, but they were not identified by mass to be related to any common oligonucleotide impurities [336,363]. By MS, only background ions were detectable for each impurity, above the lowest detectable mass of 600 g mol⁻¹. Therefore, it is likely that these impurities are small molecules <600 g mol⁻¹, perhaps due to incomplete removal of the protecting groups after global deprotection. Although these impurities decrease the purity of the final product, the significant difference in size and elution time means that these impurities are relatively easy to remove, in order to improve the purity of the final product.

The measured UV purity of membrane enabled defined sequence polymer synthesis was high, however, additional analysis of the MS purity is essential. The UHPLC method cannot resolve n-1 and n+1 impurities by UV, and these impurities can only be quantified by investigating the MS purity [360,364,365]. The MS purity of the globally deprotected oligonucleotide was inevitably much lower, 77.1%, and the overall purity corresponded to 75.2%. The MS signal is shown in **Fig. 7.10c** and the common impurity peaks are summarised in **Fig. 7.10d**.

Achieving a low concentration of n-1 impurities is essential to verify the success of the synthesiser design for membrane enabled LPS. Although n-1 impurities can form through many mechanisms, one of the problems with performing the reactions inside the retentate loop of the synthesiser is the formation of n-1 impurities through poor mixing, bypass flow, or unmixed zones in the synthesiser. For the 20-mer, 3.5% n-1 deletion errors and 0% n-2 deletion errors were identified in the final product, which corresponded to a high coupling efficiency of 99.82%. This proves that the mixing pattern inside the synthesiser was highly effective, and the volume of bypass zones and dead zones was negligible. This validates the work on mixing patterns which was completed in **Section 7.3.2**.

The low concentration of n-1 impurities also proved the success of the hub optimisation. One important consideration of the hub optimisation was maintaining a high PEG concentration which could solubilise the growing oligonucleotide. If the oligonucleotide was insoluble, the reactions would be slower, and a higher concentration of deletion impurities would form. Therefore, the low n-1 concentration proves the solubilising benefits of using a PEG based hub for oligonucleotide synthesis. The combined effect of the fast liquid phase reactions, efficient mixing, and solubilising hub resulted in membrane enabled liquid phase oligonucleotide synthesis producing a lower concentration of deletion errors than the common concentration of deletion errors achieved through SPS [4].

However, in membrane enabled liquid phase oligonucleotide synthesis 3.5% n-1 was still formed. This could be due to a number of reasons. One reason for the n-1 could be due to concentration polarisation. Concentration polarisation was shown to be significant in the synthesiser. This concentrated layer which forms on the membrane surface may be slow to fully redisperse for the reactions, resulting in a small, concentrated oligonucleotide layer remaining on the membrane surface. In this concentrated layer, the reactions would be much slower due to the slow diffusion of reactants, high viscosity, and poor mixing. Therefore, increasing the cross-flow rate for the reactions in the synthesiser is essential to increase the local turbulence and disperse this layer faster, potentially reducing the amount of n-1 formation. This is an important improvement for future oligonucleotide syntheses.

The n-1 could also be formed due to the chemistry of the oligonucleotide synthesis. Coupling efficiencies up to 99.95% have been reported in liquid phase [332]. However, this may require an increased number of building block equivalents or a longer reaction time to achieve coupling efficiencies in this range. A further reason for n-1 could be due to incomplete detritylation, or retritylation. This may be preventable by using a larger excess of acid, a stronger acid, or a larger excess of the cation trap [332]. Each of these hypotheses requires further experimentation to determine the true cause of the n-1 impurities, and to determine methods of reducing the rate of n-1 impurity formation.

Another significant impurity in oligonucleotide synthesis are addition errors, such as n+1. The main cause of n+1 impurities is commonly due to detritylation of the building block during the chain

extension reaction before the quench. This enables two monomers to be added in the same chain extension reaction. N+1 impurity formation often depends on the acidity, contact time, and concentration of the mildly acidic activator [360,363,366]. For membrane enabled oligonucleotide synthesis, only 1.2% n+1 and 0% n+2 was formed, which corresponded to 0.06% n+1 formation per chain extension. This is a low concentration of n+1 impurity, which verifies that the controlled addition of the activator to the synthesiser was successful for minimising local high activator concentrations and minimising the n+1 impurity formation, shown in **Section 7.3.2**.

A final identified impurity was P=O impurities, accounting for 12.1%. The P=O impurity is due to a single phosphodiester on the oligonucleotide backbone, rather than a phosphorothioate. This is hypothesised to be due to the presence of oxidising species in the synthesiser which oxidises the oligonucleotide before the sulphur transfer with XH. This error significantly decreases the final product purity and further work is essential to identify the cause of this impurity and reduce its formation. Critical areas for investigation are to reduce the time of the chain extension reactions, and to reduce the concentration of oxygen inside the synthesiser, with the presence of oxygen known to slowly form phosphodiesters [25,332]. Therefore, the consequence of a more effective nitrogen or argon blanket, and thorough degassing of the solvent prior to addition into the synthesiser, should be investigated.

Finally, a number of the close eluting impurities could not be fully identified by mass, 6.1%. These impurities have a mass related to the full-length oligonucleotide, with >99% of these impurities between -34.0 Da and +149.2 Da, relative to the mass of the full-length product. However, these impurities do not fall into a category of well-known impurities for oligonucleotide synthesis and their exact structure was not identifiable [336]. A significant amount of further work is required to identify each of these impurities and determine a method for reducing the formation of these impurities.

Overall, even with a significant amount of P=O impurities and significant unidentified impurities, the final product purity and yield were comparable to SPS [4,24,361,362]. Although there is a lot of further work that is required to improve membrane enabled DSP synthesis, this work shows that membrane enabled liquid phase synthesis can synthesise pharmaceutical length oligonucleotide at a yield and a

purity comparable to the current industrial standards. This is possible due to the efficient membrane separations which were shown to fully remove the building block and maintain a high hub rejection at every stage of the synthesis.

7.4 Conclusions

This work showed the successful construction of a single-phase one-pot oligonucleotide synthesiser using organic solvent separation membranes. The synthesiser was designed to minimise the volume of dead zones and bypass zones which result in impurity formation, and the mixing pattern inside the synthesiser was verified using tracer experiments. A soluble hub was then optimised to have moderate loading capacity, high solubility, and high membrane rejection. The optimal choice was a 4-arm 40 kDa PEG-based hub, when combined with the PBI membranes developed in the preceding chapters of this thesis.

Using the 4-arm 40 kDa PEG-based hub, a 20 monomer pharmaceutically relevant oligonucleotide was synthesised at moderate yield (50%) and good purity (75.2%). This was achieved in a single apparatus synthesiser in the first entirely single-phase oligonucleotide synthesis. This was facilitated by effective separations through PBI membranes. However further work is required to prolong the lifetime of the membranes, so they have consistent performance over a full 20-mer synthesis. The final 20-mer was manufactured at a yield and purity comparable to SPS, and a number of methods of improving the yield and purity of membrane enabled liquid phase synthesis were discussed. With these improvements, and the inherent automatability and scalability of this work, it is believed that this manufacturing technique may surpass the conventional industrial technique of SPS for the synthesis of oligonucleotide DSPs.

8.0 Conclusions and Future Work

8.1 Conclusions

To manufacture defined sequence polymers (DSPs) using liquid phase synthesis (LPS), a high purity and high yield separation technique is essential. Compared to other DSP purification techniques, such as precipitation and extraction, membrane separations in organic solvents are highly effective, efficient, and can be automated and scaled to different manufacturing requirements. Previous studies showed that membrane enabled liquid phase DSP synthesis is viable [99]. However, the separations were inefficient, leading to high solvent consumption, low yields, and significant concentrations of impurities in the final DSP [29]. In this thesis, through the development of a new series of membranes and a new DSP synthesiser, membrane enabled liquid phase DSP synthesis was shown to be a successful method of synthesising industrially relevant length DSPs. If commercialised, this technology could provide a scalable and high purity method of manufacturing DSPs, rivalling the current industry standard of solid phase synthesis (SPS).

To manufacture DSPs using this platform technology, a new range of membranes specifically targeting the purification of DSPs was required. In **Chapter 4**, PBI membranes were manufactured and crosslinked with DBX. The crosslinked membranes were shown to be stable in a wide range of solvents including strong polar aprotic solvents (DMF and DMAc), acids ($\text{pH} < 0$), and bases ($\text{pH} > 0$). After crosslinking, the PBI membranes were graft modified with a linear polyetheramine (1kPEG) and a competitive reaction with 2-MEA was used to control the extent of 1kPEG modification. By controlling the extent of modification, the molecular weight cut off (MWCO) was customisable between tight ultrafiltration and loose nanofiltration membranes, with a higher extent of modification resulting in a tighter membrane. This was shown to be due to graft modification inside the permeation pathways of the membrane, not just modification of the top surface. In acetonitrile these membranes showed efficient permeation of molecules between 500 and 2,000 g mol^{-1} , while retaining molecules between 2,000 and 20,000 g mol^{-1} . This makes these membranes ideally suited to the synthesis of DSPs where the size of the monomers and reaction debris are less than 2,000 g mol^{-1} .

Also in **Chapter 4**, the casting, crosslinking, and graft modification reactions were scaled to 0.3 m x 5 m in area, and the scaled-up membrane was used to produce spiral wound modules. The modules showed consistent rejection profiles to the flat sheet membranes. This demonstrated the uniformity of the membrane crosslinking and modification reactions, and the potential for industrial scale manufacture of graft modified PBI membranes.

In **Chapter 5**, the physical stability of the PBI membranes was evaluated. Almost all organic solvent stable PBI membranes in literature are based on a 0.7 IV PBI solution ($\sim 27,000$ Da). However, when continuously cast at 18 wt%, this membrane was observed to be susceptible to polymer chain rearrangement and densification of the selective layer, even after crosslinking with dibromoxylene (DBX). Although the 0.7 IV membranes were chemically stable, the membranes were found to not be process stable, and the densification was enhanced by using strong polar aprotic solvents, such as DMF, at high temperature. Therefore, to improve the physical stability of PBI membranes, a custom 1.1 IV PBI solution ($\sim 60,000$ Da) was used. Manufacturing membranes with a high molecular weight PBI polymer led to a significantly lower flux for the same molecular weight cut-off (MWCO). However, by changing the composition of the dope solution, PBI membranes with a comparable flux or rejection to the low molecular weight PBI could be manufactured. After the same extent of crosslinking with DBX, the higher molecular weight PBI exhibited consistent permeance over long-term studies, even in strong polar aprotic solvents at high temperature. This phenomenon was attributed to a higher degree of interchain interactions and chain entanglement in the high molecular weight polymer. This reduced the chain mobility and increased the process stability of the PBI membrane. This resulted in consistent long-term membrane performance, which is essential for DSP synthesis.

In **Chapter 6**, solvent stable high molecular weight PBI membranes were graft modified with three different modifiers, 1kPEG, 2kPEG, and 2kPPG, and the anti-fouling properties of these membranes were tested with colloidal silica particles as a model for fouling system. Graft modification of the membranes was shown to be a highly effective method of producing anti-fouling organic solvent stable membranes. Additionally, the anti-fouling properties were shown to be related to the size of the graft

modifier in solution. A larger modifier created a larger steric barrier which prevented the foulant reaching the surface of the membrane, therefore creating a membrane with improved anti-fouling properties. However, the size of the steric barrier was significantly dependant on the process solvent. In polar organic solvents, hydrophobic PPG-based modifiers were found to be more effective than an equivalent molecular weight PEG-based modifier. This was due to preferential solvent-PPG interactions which resulted in the PPG modifier having a larger hydrodynamic diameter in solution. This was contrary to the common results in aqueous systems, where PEG-based modifiers show excellent anti-fouling properties, and PPG-based modifiers show no anti-fouling benefit due to unfavourable water-PPG interactions. In non-polar solvents, both the PEG and PPG based modifiers were observed to have unfavourable solvent-modifier interactions which resulted in the modifier collapsing to a much smaller hydrodynamic diameter in solution, therefore providing minimal anti-fouling effect. By tuning the choice of graft modifier to favour preferential interactions with the process solvent, the hydrodynamic diameter of the modifier was increased, and the anti-fouling properties were maximised. Hence, anti-fouling membranes can be specifically designed for the polar solvents which are used in DSP synthesis.

In **Chapter 7**, a pharmaceutically relevant length oligonucleotide was synthesised to show the commercial potential of membrane enabled liquid phase DSP synthesis. This was possible due to the PBI membranes developed in the previous chapters of this thesis. A DSP synthesiser was designed to have efficient mixing and to perform both the reaction and separation steps in a single apparatus. This removed the requirement for material transfer, which is time consuming and difficult to automate. The DSP synthesiser was combined with a soluble hub, which was optimised to achieve a moderate loading capacity and maximise the hub rejection, specifically at high concentrations where mass transfer and concentration polarisation were significantly influencing the permeance and hub rejection. This enabled efficient liquid phase purification, exclusively using PBI membranes, and resulted in the synthesis of the first oligonucleotide of pharmaceutically relevant length (20-mer) using membrane-based purification. The final product had moderate yield (50%) and good purity (75.2%), comparable to SPS. Additionally, a number of potential improvements were outlined, which could be used to improve

membrane enabled liquid phase DSP synthesis. Some of these are discussed in **Section 8.2** as the important next steps of this work.

8.2 Future Work

The moderate yield and good purity of the final oligonucleotide showed that membrane enabled DSP synthesis has potential as a manufacturing platform for industrial DSP manufacture. However, significant further work is required before membrane enabled DSP synthesis can complete with, or surpass, SPS. This further work is divided into four separate sections. These sections focus on improvements to the membranes, synthesiser, hub, and DSP synthesis.

8.2.1 Membrane Development

The PBI membranes developed in this thesis enabled the highly efficient purification of a wide range of DSPs. This was partly possible due to graft modification, which was used to tailor the membrane performance. In this work PEG and PPG was selected as the graft modifiers because they are cheap, industrially available, and chemically inert. However, there are a number of different graft modifiers which could be used to tailor the membrane performance and improve the rejection of oligonucleotide hubs. Graft modifiers of interest include hydrophobic, branched, and charged polymers. This development work is highly exploratory. However, by using the grafting methodology established in this work, different graft modifiers could be tested to further improve the performance of the membranes for DSP synthesis.

8.2.2 Synthesiser Development

For oligonucleotide synthesis, a novel synthesiser was designed to reduce dead zones, promote good mixing, and reduce membrane fouling. However, there are a number of further areas of synthesiser development which are essential to further improve membrane enabled DSP synthesis.

The first area of development is the implementation of automation. One of the major advantages of SPS is the speed of manufacture. This is partly due to the significant process automation which has been developed for SPS. In this work, one of the important achievements was the first liquid phase synthesis of an oligonucleotide in a single apparatus synthesiser without material transfer. This enables the

process to be fully automated, analogous to SPS. However, in the constrained time frame of this work, significant automation could not be implemented. The introduction of automated reagent dosing, and automated transitions between reaction steps and separation steps, will enable the synthesiser to be operated with less operator requirements, resulting in a faster, more reliable, and more efficient process, which is more comparable to the speed of SPS.

A second important area of further synthesiser development is the implementation of inline or online monitoring to the synthesiser. In this work, the reactions and separations were monitored atline by manually taking samples throughout the DSP synthesis and analysing with UHPLC. The implementation of inline UV, IR, or UHPLC monitoring will reduce the manual labour requirement for sampling and provide a large amount of continuous process data. This can be used to ensure that all process steps reach completion, predict sequence complications, and increase the product purity. Additionally, inline monitoring can be linked to the process automation. This can be used to ensure that a target criteria is achieved for every process step before starting the next process step. This is used in some steps of SPS, but with the advantage of liquid phase reactions, monitoring and automation can be performed for every single step of LPS. Therefore, it is predicted that inline monitoring will significantly improve the efficiency, yield, and purity of membrane enabled liquid phase DSP synthesis.

The third important area of further synthesiser development is to increase the manufacturing scale of oligonucleotide synthesis by implementing spiral wound modules into the synthesiser. This is essential to prove the scalability of the process and show that the process can be industrialised. In **Chapter 4**, PBI spiral wound modules were shown to have consistent performance with flat sheet cells. However, using a module for DSP synthesis requires a customised module design to ensure good mixing and no bypass zones. Additionally, an investigation into the effect of different feed spacers is essential to maximise the local turbulence and minimise the concentration polarisation inside the module. Finally, the synthesiser will need to be upgraded to have a comparable cross-flow velocity to that of the flat sheet cells, ensuring that concentration polarisation is not significantly increased on scale up.

8.2.3 Hub Development

A PEG based hub was selected for this work after evaluation of the previous work on liquid phase oligonucleotide synthesis. This was due to the improved solubility of PEGylated oligonucleotides in acetonitrile. However, using a different hub molecule can increase the hub loading and change the hub-solvent-membrane interactions, therefore improving the rejection of the hub. Hub designs of interest include using rigid cores, charged hubs, or an increased number of arms. This optimisation requires a high throughput screening of many different molecules, with many different membranes. The final hub choice will have a significant impact on the economics of the process and can only be selected by conducting a comprehensive economic analysis, focusing on minimising the cost of the final product.

8.2.4 DSP Synthesis Development

A number of improvements must also be made to the DSP synthesis methods. The first requirement is to prolong the lifetime of the membranes, so they have consistent performance for a minimum of one full synthesis, and preferably for multiple consecutive syntheses. The loss of membrane performance was predicted to be due to fouling. Therefore, a thorough investigation into the nanoparticles which are present in all reagents is essential to remove the fouling at source. Additionally, the application of anti-fouling membranes, such as those developed in **Chapter 6**, will prolong the lifetime of the membranes, and significantly improve the synthesis yield.

Further work is also needed to improve, develop, and optimise the chemistry of oligonucleotide synthesis for liquid phase reactions. This platform technology was tested with the synthesis of a 2'-methyl RNA phosphorothioate 20-mer. However, there were a significant number of impurities present in the final product. Further work is needed to evaluate the formation mechanism of each of these impurities and develop a methodology to reduce its formation. For example, a significant quantity of P=O error was present in the final product. This was attributed to competitive oxidation in the coupling reaction. Further work is needed to identify if this was indeed the cause of the impurities and determine methods of reducing the rate of oxidation.

For the successful manufacture of oligonucleotide therapeutics, this platform technology must also be tested using phosphodiesteres, and phosphoramidites with other chemical modifications, which are essential in oligonucleotide therapeutics. Additionally, to investigate membrane enabled liquid phase DPS synthesis as a new platform technology, the effectiveness of this technology for the synthesis of other DSPs needs to be investigated.

The final critical step of future work is a thorough economic evaluation of the industrial prospects of membrane enabled liquid phase DSP synthesis. Currently, this process is expensive due to the high reagent consumption, labour requirement, and expensive hub molecules. However, with automation, economies of scale, optimisation of the reagent quantities, and optimisation of the hub, the cost of this process will decrease. Therefore, after the improvements to the membranes, synthesiser, hub, and DSP synthesis process, a thorough cost comparison to SPS is essential.

If the further work described in this chapter is successful, and the economics of this process are shown to be favourable, membrane enabled liquid phase synthesis could become a cost effective, high purity, and high yield method of manufacturing a wide range of DSPs. This would be made possible due to the foundation work completed in this thesis, and it is predicted that this platform technology will be significant for the pharmaceutical sector, polymer science, information storage, and countless other industries.

9.0 References

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38	2.5	J.F. Kim, P.R.J. Gaffney, I.B. Valtcheva, G. Williams, A.M. Buswell, M.S. Anson, A.G. Livingston, Organic Solvent Nano filtration (OSN): A New Technology Platform for Liquid-Phase Oligonucleotide Synthesis (LPOS), (2016). https://doi.org/10.1021/acs.oprd.6b00139 .	ACS Publications	11/08/22	Yes
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