Organic solvent nanofiltration for microfluidic purification of poly(amidoamine) dendrimers

Jack T. Rundel\textsuperscript{a}, Brian K. Paul\textsuperscript{b}, Vincent T. Remcho\textsuperscript{a,∗}

\textsuperscript{a}Department of Chemistry, Oregon State University, Corvallis, OR 97331, USA
\textsuperscript{b}Department of Industrial and Manufacturing Engineering, Oregon State University, Corvallis, OR 97331, USA

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Abstract

A nanofiltration method has been developed in a microfluidic format for the continuous-flow pressure-driven purification of half-generation poly(amidoamine) (PAMAM) dendrimers, a family of macromolecules characterized by highly branching structures radiating from a central core, without additional solvents or buffers. An organic solvent resistant nanofiltration membrane, STARMEM 122, has been fully integrated into a hard polymer microfluidic module by transmission laser welding. The membrane was initially characterized in a bench-top test fixture to determine the solvent permeance and percent rejection of a surrogate molecule, Rhodamine B, at lower than typical operating pressures ($P < 7$ bar). The microfluidic module then underwent similar testing at 1.4 bar with the surrogate and with the generation-0.5 PAMAM dendrimer. This approach to nanofiltration will readily interface to upstream microreactors.

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1. Introduction

Over the past few decades, nanofiltration methods have been developed on the macroscale for applications such as wastewater purification and food production. Organic solvent resistant polymeric membranes with low molecular weight cutoffs (MWCO < 1000) have recently become commercially available and applied in areas such as the recycling of lubricating oils [1]. Similar techniques, implemented on the microscale, offer tremendous potential for application in pharmaceutical and bioanalytical industries where product purification methods are needed with minimization of solvent volume in both aqueous and non-aqueous environments. Of particular interest is the development of microreactors for on-demand synthesis of nanoparticles and macromolecules in which reduced residence times can be realized for diffusion-limited parameters such as mixing of reagent streams and heat exchange.

However, product purification in a microfluidic format presents significant challenges. Traditional methods, such as solvent vaporization, are impractical, environmentally unsound and energy intensive. Most popular preparative chromatographic methods, such as solid-phase extraction, are limited to batch mode and do not address higher throughput needs. Continuous separations are possible via simulated moving bed chromatography (SMB) and centrifugal counter-current chromatography (CCC). However, these methods will not scale easily to the microfluidic format.

In this study, an organic solvent resistant polyimide nanofiltration membrane, STARMEM 122 (Membrane Extraction Technologies, London, UK), has been integrated into a microfluidic module and characterized for product purification following on-chip synthesis of half-generation poly(amidoamine) (PAMAM) dendrimers. The method allows continuous-flow pressure-driven product purification in a microfluidic format without additional solvents or buffers. Initial characterization of the membrane was achieved in a bench-top test fixture to determine the permeance of solvents and percent rejection of a surrogate molecule at lower than typical operating pressures ($P \leq 7$ bar).

Dendrimers, with a high degree of monodispersity and spherical symmetry, have been used previously to characterize pore size and Donnan exclusion effects in polymeric membranes [2]. Organic solvent resistant nanofiltration membranes have been used on the macroscale in solvent exchange and homogeneous-catalyst recycling following organic syntheses [3]. Polyimide
filtration membranes have been fabricated in situ for use in microfluidic systems [4]. To the authors’ knowledge this is the first report of incorporating a polymeric filtration membrane into a hard polymer microfluidic device by transmission laser welding.

2. Theory

2.1. Nanofiltration

In filtration processes, the fraction of the feed stock which passes through the filter is the permeate whereas the fraction which is rejected by the filter is known as the retentate. A filtration process is typically characterized by a molecular weight cutoff (MWCO) at which 90% rejection occurs for species of that molecular weight Eq. (1). Nanofiltration has been defined as a “pressure-driven membrane-based separation process in which particles and dissolved macromolecules smaller than 2 nm are rejected” [5] and also as having a MWCO range of 200–1000 [6].

Rejection, \( R = 1 - \frac{C_p}{C_r} \)  

In nanofiltration processes percent rejection has been observed to decrease at lower pressures [7]. Percent rejection and permeance are also highly dependent upon the solvent and solute conditions [8,9]. This is thought to be due to solute/solvent, solute/membrane and solvent/membrane interactions that are not easily predictable. As a result, nanofiltration membranes must be evaluated empirically for differing solvent systems and pressure ranges.

2.2. PAMAM dendrimers

Poly(amidoamine) dendrimers are a family of macro-molecules characterized by highly branching structures radiating from a central core. A brief synopsis of the divergent synthesis of PAMAM dendrimers with an ethylene diamine (EDA) core is provided here in order to provide context for the gent synthesis of PAMAM dendrimers with an ethylene diamine radiating from a central core. A brief synopsis of the divergent molecules characterized by highly branching structures is handled properly. Nanofiltration offers an elegant alternative to this process provided adequate mass discrimination between the excess reagents and the desired product.

3. Experimental

3.1. Reagents and materials

The organic liquids used were: methanol (MeOH) (HPLC grade, Merck, Whitehouse Station, NJ, USA) as solvent; methyl acrylate (>99.0%, Fluka, Sigma–Aldrich, St. Louis, MO, USA) as excess reagent for PAMAM half-generation synthesis; ethylene diamine (Fisher, Pittsburgh, PA, USA) as excess reagent for PAMAM full-generation synthesis; and glacial acetic acid (HAc) (Fisher) as a potential protonating agent for EDA for PAMAM full-generation synthesis; and glacial acetic acid (HAc) (Fisher) as a potential protonating agent for EDA

3.2. Materials compatibility

A survey of possible polymer candidates was conducted to identify a substrate that would not be attacked by the various solvent environments. The ideal hard polymer substrate would
be: (1) thermoplastic for future embossing of microfeatures; (2) transmissive in the visible for optical monitoring of the filtration process and transmissive in the near infrared (NIR) for transmission laser welding; (3) chemically resistant to the various organic solvent environments; and (4) machinable with minimal burring to allow for rapid prototyping in the design phase. Hard polymers selected for study included APET, PSU, PVC, PET, PETG, PEI, PC, PEEK, PMMA. Elastomeric polymers selected for study included PDMS, Viton and EPDM.

Polymer samples of interest were cut to size at 0.25 in. × 1.5 in. Solvent environments of 100, 10 and 1% (v/v) dilutions in MeOH of EDA, MA and HAc were prepared in bulk and dispensed as 2.0 ml aliquots into 4 ml glass vials. Samples of each polymer were massed and placed in the respective solvent environments. A control for each polymer type, not exposed to the solvent environment, was left at ambient atmospheric conditions in a sealed plastic bag for the duration of the test. After 24 h exposure each sample was removed from the solvent environment, rinsed with MeOH to remove any residual solvent, and blown dry with N2 gas to remove any residual MeOH. The samples were then weighed, placed in rough vacuum for 10 min and weighed again. The net loss or gain in mass was then calculated. Visual inspection of the polymer samples provided qualitative observations including haze, swelling, discoloration and etching. Each sample was then returned to its respective solvent environment for long-term exposure observations.

The performances of PET, PSU, STARMEM 122 (SMA) and PEEK are shown in Fig. 1. PMMA, PC, PEI, PVC, PETG, PDMS and Viton were rejected due to excessive changes in mass due either to solvent uptake or dissolution of the polymer. PVC, PETG and PDMS also showed unacceptable discoloration and haze. PET was found to be the best hard polymer substrate. Optically transparent PET is widely available in sheets thinner than 1 mm and ubiquitous as beverage containers and food packaging. However, due to its semi-crystalline structure, larger thicknesses are only available in the naturally white-opaque form. As a result, APET, available in optically transparent 1 mm sheets, was selected as the hard polymer substrate for the microfluidic module. PSU was selected for the macroscale test fixture because APET was not available in the required 0.5 in. thickness.

STARMEM 122 was found to be compatible with MA and incompatible with EDA. Consequently, although this method is suitable for half-generation synthetic steps, in its current form it cannot be applied to purification following the synthesis of full-generation PAMAM dendrimers. Alternatively, ceramic membranes, available with excellent chemical compatibility and reported MWCO = 450 (Inopor, Hermsdorf, Germany) could be used in place of the polymer membrane. However, ceramic membrane thicknesses are typically limited to >800 μm making them less attractive for microfluidic applications. The extremely high melting points of most ceramics eliminate the possibility of welding them to polymer substrates. Therefore, the strategy described here may not prove viable for integration of ceramic nanofiltration membranes into a microfluidic format. A second approach would be a post-synthetic protonation of the amine-terminated EDA and full-generation PAMAM dendrimers via the addition of an acid to the product stream. An organic acid, such as acetic acid, would be expected to filter out along with the excess protonated-EDA. However, the dendrimer product would need to be de-protonated prior to the next synthetic step. This would likely require the addition of yet another species to the product stream that would either be tolerated as a bystander during the subsequent syntheses or somehow removed by yet another process. It is hoped that a polymeric nanofiltration membrane will be developed soon to withstand exposure to EDA yet remain amenable to transmission laser welding.

3.3. Fluidic control

Both the macroscale test fixture and microfluidic nanofiltration module were interfaced with the same fluidic control system (Fig. 2). For tests with the macroscale fixture, the path from the
fixture to the retentate reservoir was removed and plugged to establish dead end flow. For tests in the microfluidic module, the path to the retentate reservoir was preserved and an adjustable bleed valve to atmosphere was used to control a pressure drop and thereby fluid flow between the feedstock reservoir and retentate reservoir. The feedstock and retentate reservoirs were pressurized by supplying nitrogen gas to the headspaces. The pressure was adjusted and monitored at the tank cylinder regulator. Capillary rinse kits (Sigma–Aldrich) provided reservoirs for the feed, permeate and retentate. Swagelok 1/16 in. stainless steel ball valves (Portland Valve & Fitting, Portland, OR, USA) controlled the routing of N₂ gas. Luerlock fittings and 0.015 in. I.D. × (1/16) in. O.D. PEEK tubing (Upchurch Scientific, Oak Harbor, WA, USA) were used for all fluidic connections between components.

3.4. Macroscale test fixture

A macroscale test fixture (Fig. 3), constructed of 0.5 in. PSU, was machined using a shop lathe and mill. The fixture was designed to provide a 10 cm² membrane surface area and 1 mm channel height above the membrane. The fluidic interface to the macroscale test fixture was comprised of 1/4 in.-28 Delrin fittings and Tefzel ferrules (Upchurch Scientific). The membrane and backing material were cut to size with a cylindrical stainless steel punch fabricated specifically for that purpose. Gasketing was achieved with a PTFE-encapsulated Viton O-ring and mechanical support for the membrane was provided by a sintered glass frit.

3.5. Microfluidic nanofiltration module

A microfluidic nanofiltration module was designed and fabricated for permeance and percent rejection testing (Fig. 4). The product stream channel was milled to a 100 μm depth in 1 mm thick APET with an inlet for the feed stream and an outlet for the retentate. A counter flow channel was milled to a depth of 250 μm into a second 1 mm thick APET substrate with an inlet for the sweep and an outlet for the permeate. The channel was completely filled by a fibrous backing layer providing mechanical support to the membrane as well as a flow path for sweep and permeate. The resulting geometry provided 4 cm² surface area available for filtration.

The STARMEM 122 sheet, cut with scissors into a rectangular blank with dimensions larger than the product stream channel, was laser welded to the APET substrate with the active layer of the membrane toward the product stream channel. This formed a gasketing seal around the entire perimeter of the product stream channel. The excess membrane was removed by peeling away the area exterior to the gasketing weld thereby achieving a clean edge. However, this peeling process delaminated a backing layer component of the membrane which normally provides mechanical support.

In order to achieve a uniform structural weld, a crush zone with a height of 50 μm and width of 1 mm was milled along the entire perimeter of the counter flow channel. The uppermost surface of the crush zone provided the only contact between the two APET substrates thereby limiting the clamping force to this region. During laser welding, the crush zone of the lower substrate melted along with the area of the upper substrate it was in contact with. The applied clamping pressure forced the two substrates together promoting fusion of the structural weld upon cooling.

All milling was performed on a Tool Crafter mill (CMS CNC, Laguna Hills, CA, USA). The fibrous backing layer was cut to size using an ESI 5330 (Electro Scientific Industries, Portland, OR, USA) laser trimming system. The entire assembly was gas-ketated and structurally bonded via transmission laser welding following the deposition of Clearweld LD120B (Gentex, Zeeland, MI, USA) along the weld lines. The laser welding was performed with an IRAM 300 (Branson Ultrasonics, Danbury, CT, USA) laser welding system retrofitted with a 940 nm vertical stack diode array dispersed in a 1.8 mm × 80 mm swath. For the membrane-to-APET weld, the diode array was supplied with a current of 40.0 A resulting in 195 W of photonic power. For the APET-to-APET weld the diode array was supplied with a current of 50.0 A resulting in 275 W of photonic power. Both welds
were realized with a scan speed of 10 mm/s and a clamping force of 1.3 kN (4.1 bar on a 64 mm diameter air cylinder).

A fluidic interface fixture for the microfluidic module (Fig. 4) was designed and fabricated to register the microfluidic module inlets and outlets and to provide clamping support for the O-ring gaskets. The fluidic interface was comprised of Nanoport assemblies to accept 0.015 in. I.D. × (1/16) in. O.D. PEEK tubing (Upchurch Scientific) and M1x4 EPDM O-rings to provide solvent resistant gasketing of the nanoports. The fixture was constructed from 0.5 in. PC and stainless steel machine screws.

The microfluidic module was originally designed for a counter current flow in which pure methanol flows in the downstream channel to sweep away the permeate (Fig. 5). However, the module was operated in a cross-flow geometry in order to sample the permeate without dilution from a sweep stream and thereby more accurately assess the performance of the membrane. Future process development will move toward implementation and characterization of the counter current flow operational mode.

3.6. Permeance and percent rejection measurements

Prior to initial use, each membrane was rinsed a minimum of 10 times for 20 min at 1.4 bar in dead end flow until a stable flux was observed. This preconditioning step is necessary to remove an impregnating oil that stabilizes the dry membrane during shipment and storage.

Masses of the permeate and retentate were found by the mass difference between the final and initial masses of the respective reservoirs. Volumetric flux was calculated by the product of mass flux and fluid density. For cross-flow modes, retentate and permeate volumetric flow rates were held equal by adjustment of the bleed valve.

Rhodamine B was used as a surrogate for \( G^{-0.5} \) (MW = 404) due to ease of detection, a similar molecular weight (MW = 479), high solubility in MeOH, and stability at standard conditions. The concentration of RhB was determined with a Luminescence Photometer (Perkin-Elmer, Waltham, MA) scanning from 350 to 700 nm with a fixed 25 nm offset between excitation and emission wavelengths and 5 nm excitation and emission slit widths. The retentate and permeate signals were taken at 543 nm due to the high signal strength and signal to noise ratio in that region (Fig. 6).

\( G^{-0.5} \) was tested in the microfluidic module at a 1 mg/ml concentration in 4 mM MA in MeOH. These concentrations represent the expected relative ratios of product and excess reagent following half-generation synthesis. However, the absolute con-
centrations are approximately two orders of magnitude more dilute than published values [10].

Absorbance of G—0.5 was scanned against a blank of 4 mM MA on an Agilent 8453 UV–Visible Spectrophotometer (Agilent, Santa Clara, CA) from 190 to 400 nm using a glass cuvette with a 1 cm path length. The permeate signal was seen to be higher than expected possibly due to an unidentified interferrent. As a result, the relative concentration of G—0.5 in the permeate was calculated using the absorbance signal of the feedstock and retentate Eq. (2), where C is the concentration; I the absorbance signal; V the volume; and f, p, r indicate feed, permeate, retentate. Percent rejection was reported as an average of percent rejections calculated in the range of 225–230 nm due to high signal strength and SN in that region.

\[
I_p = \frac{(V_p + V_r)I_f - V_rI_f}{V_p} 
\]

\[
mol_f = mol_p + mol_r \tag{2a}
\]

\[
V_fC_f = V_pC_p + V_rC_r \tag{2b}
\]

\[
V_f = V_p + V_r \tag{2c}
\]

\[
C_i \propto I_i \tag{2d}
\]

4. Results and discussion

4.1. Analysis

The microfluidic module was limited to a pressure of 1.4 bar due to leakage through the membrane observed at higher pressures. This was most likely a consequence of module fabrication since the membrane itself is normally operated at 30–60 bar and no leakage was observed in the macroscale test fixture at pressures up to 7 bar. Possible factors contributing to the failure include: (1) removal of the backing material following the laser welding of the membrane to APET substrate; (2) reduction in membrane tensile strength along the gasketing weld due to heat effects of the welding; (3) a compression of the fibrous backing material filling the counter flow channel resulting in a bowing of the membrane into the counter flow channel and consequently an increase in the tensile and shear forces at the gasketing weld line; and (4) an observed outward bowing of the APET substrate of the bottom of the counter flow channel resulting in an increase in the tensile and shear forces at the gasketing weld line. Efforts are underway to analyze and remedy these possible failure modes.

The flux of the permeate across the membrane was found to be highly linear with pressure in both the macroscale test fixture and in the microfluidic nanofiltration module (Table 2). The slope of flux versus pressure describes the permeance of a given solvent environment while the intercept describes the theoretical flux at zero applied pressure. For applications on the microscale, units of \( \mu L, cm^2 \) and min offer a more relevant treatment. For example, a volumetric flow rate of \( 110 \mu L \text{ min}^{-1} \) was observed for the permeate of a 10% (v/v) MA feedstock at 1.4 bar across the 4 cm\(^2\) of membrane surface area in the microfluidic module.

Permeance in the macroscale test fixture was found to be greatest for pure MeOH and to decrease with the addition of RhB and MA. Permeance in the microfluidic module at 1.4 bar shows a similar trend but with higher overall values. These permeance values of \( \sim 14–16 L m^{-2} h^{-1} bar^{-1} \) are somewhat greater than the 2–3 L m\(^{-2}\) h\(^{-1}\) bar\(^{-1}\) for MeOH across STARMEM 122 at 30 bar reported in the literature [9].

Percent rejection for \( 10^{-7} \) M RhB at 1.4 bar was found to be 55.7 ± 0.9% for dead end flow in the macroscale test fixture and 60.6 ± 1.8% for cross-flow in the microfluidic module. These values are significantly lower than the >90% rejection expected for RhB under normal operating conditions of pressures ranging from 30 to 60 bar. Percent rejection versus pressure data in the macroscale test fixture shows a positive correlation from 1.4 to 6.9 bar obtained using dead end flow (Fig. 7). Percent rejection

### Table 2

Average permeances (L m\(^{-2}\) h\(^{-1}\) bar\(^{-1}\)) of various solvent environments in the macroscale test fixture and the microfluidic module

<table>
<thead>
<tr>
<th>Solvent environment</th>
<th>Macroscale test fixture average permeance</th>
<th>Microfluidic module average permeance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>13.6 ± 0.2</td>
<td>16.2 ± 1.1</td>
</tr>
<tr>
<td>( 10^{-7} ) M RhB:MeOH</td>
<td>13.1 ± 0.1</td>
<td>17.4 ± 0.9</td>
</tr>
<tr>
<td>10% (v/v) MA:MeOH</td>
<td>8.1 ± 0.1</td>
<td>12.4 ± 0.7</td>
</tr>
</tbody>
</table>

Macroscopic pressure range 1.4–6.9 bar, standard errors shown for slope and intercept of volumetric flux vs. pressure. Microfluidic pressure fixed at 1.4 bar with standard deviation shown for the average permeance.
Fig. 7. Percent rejection vs. transmembrane pressure of $10^{-7}$ M RhB in MeOH in the macroscale test fixture showing a slightly positive correlation. A minimum of three data points were used for each data set. Error bars are ±1 standard deviation.

for G−0.5 at 1 mg/ml and 4 mM MA in the microfluidic module was found to be 55.2 ± 2.3%.

4.2. Discussion

In this filtration process, low MW species such as MeOH and MA are expected to move across the membrane at roughly the same rate [9]. As a result, the concentration of MA can only be decreased by iterative filtration steps in which the volume of the retentate is significantly reduced in every step and diluted with pure MeOH between steps. A theoretical treatment Eq. (3) can be developed using Eqs. (2a)–(2c) and (1); assuming $R$ is constant as the solvent/solute environment changes; and defining \( n \) = number of filtration steps. By setting \( V_f/V_r = 10 \), we find that three filtration steps would be necessary to reduce the concentration of a species with 0% rejection by three orders of magnitude (Fig. 8). We also find that those same three filtration steps would reduce a species with 60% rejection by two orders of magnitude. In fact, percent rejections >90% are necessary in order to achieve retentions >10% after three filtration steps.

\[
\frac{\text{mol}_l}{\text{mol}_r} = \left[ \left( \frac{V_f}{V_r} - 1 \right) (1 - R) + 1 \right]^{-n} \tag{3}
\]

Therefore, the removal of MA following the synthesis of G−0.5 is not practical with this microfluidic module at 1.4 bar due to the extensive product loss. Fortunately, G−0.5 represents a worst-case scenario for microfluidic nanofiltration of PAMAM dendrimers. Each subsequent generation is higher in mass and is expected to experience higher percent rejection under the same solvent and pressure conditions. Future work is needed to verify this expectation and to determine percent rejections for higher generations at these conditions.

However, the relatively low percent rejection for G−0.5 could be used as an advantage in purification of higher generations. Any excess MA remaining in the retentate from the preceding purification will react with EDA to produce G−0.5 as a side-product. Removal of this lower generation dendrimer is critical in order to preserve monodispersity in subsequent generations. If the higher generation dendrimer has 99% rejection, only 23% of the higher generation dendrimer is expected to be lost in the permeate.

This microfluidic nanofiltration module is a front-end device that has tremendous utility as an on-line sample preparation tool prior to chromatographic and/or electrophoretic separations on a chip. Possible applications include but are not limited to: (1) pre-concentration of high molecular mass analytes in large-volume environmental samples in the field; (2) more rapid sample prepared in the laboratory such as desalting of proteins or recovery of drugs from biological samples; (3) fractionation of molecules in flowing product streams for automated process analyses; and (4) continuous purification of product streams to facilitate nanomaterials synthesis and analysis.

5. Conclusion

Transmission laser welding of a polymeric nanofiltration membrane within a hard polymer microfluidic device has been demonstrated. The resulting module has been designed to be chemically compatible with MeOH and 10% (v/v) MA and to operate at pressures up to 1.4 bar. The microfluidic module has been evaluated for purification of the half-generations of PAMAM following synthesis in a microreactor. The first purification step for G−0.5 will likely experience significant product loss due to a low percent rejection. However, product loss is expected to decrease with higher generations due to a predicted increase in percent rejection resulting in an increase in efficiency. The relatively low percent rejection of G−0.5 might prove to be advantageous in removal of unwanted sideproducts following higher generation syntheses.

A significant barrier remains in the purification of full-generation PAMAM dendrimer due to incompatibility of STARMEM 122 membrane with EDA and, by extension, the
amine-terminated full-generations themselves. Protonation of EDA and full-generations might achieve compatibility but would likely require the introduction of other species into the product stream. Ceramic membranes provide a possible alternative with excellent chemical resistance and low molecular weight cutoffs. However, integration of relatively thick ceramic membranes into hard polymer microfluidic devices remains a challenge.

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