ANALYTICAL AND BIOMEDICAL APPLICATIONS OF POROUS MEMBRANES

ANALYTICAL AND BIOMEDICAL APPLICATIONS OF POROUS MEMBRANES

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ABSTRACT

Membrane filtration is widely used to biomedical and analytical applications. Compared to other techniques available membrane filtration provides fast processing time, easy availability, robust performance and relatively low cost. These advantages make ultrafiltration and microfiltration well integrated into bioseparation, purification of biomedical materials and downstream polishing. Apart from the advatanges, there are certain drawbacks with microfiltration and ultrafiltration. While perceived negative in many scenarios, the effects does not necessarily counteract the purpose of the process and could find some useful applications if treated from a different perspective.

By the virtue of fast processing of membrane filtrations, applications were made in processing biomedical materials and developing analytical methods. Poly(*N*-isopropylacrylamide) microgels are of potential in many biomedical applications. Microfiltration and ultrafiltration of such microgels for fast purification were explored. Meanwhile, the environmental responsive behaviours of such microgels bring about opportunity and challenge. Investigations were made on the salt-responsive transmission behaviours of microgels in microfiltrations. A hypothesis was raised and verified. Implications of applications *in vivo* were drawn based on experimental results. Many techniques for analysis of protein-drug binding have been under development. A new alternative utilizing pulsed tangential flow ultrafiltration was developed in this study and used to obtain binding data between aspirin and BSA under different conditions. The performance of the systems was assessed under different parameter settings. Possibility of further automation was discussed. On account of the fouling and concentration

polarization, a new perspective was taken with the effort of developing such effects into potential applications. Patterned fouling was introduced and the fouled membrane was used to filter coloured feed to reveal the patterns transferred. Concentration polarization in ultrafiltrations with different levels of fixation of membranes was visualized by dyed particles. The possible flow modes under these conditions were suggested. A hypothesis was attempted from a fluidics point of view.

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1. Introduction

1.1 Backgrounds

Porous membranes are thin layers with pores of sizes that fall in a narrow range. They are commonly used as filters and porous adsorbents. Membranes offer fast processing time, simple mechanism, robust performance and good accessibility. There are also disadvantage accompanying, such as fouling of membranes and concentration polarization during processes. The focus of this study is on the barrier properties of ultrafiltration and microfiltration membranes in various biomedical and analytical applications. In the progression of the body, the advantage of speed is applied in proposing alternatives to existing techniques and then the disadvantages are examined with careful discretion in seek to create potentially useful applications.

1.1.1 Biomedical applications

Membrane techniques are widely used in bio-related research and industry. Membrane filtrations in particular are known for their fast process, robust performance for commercial products, simple operation and multi-functionality with biomedical products. A wider spectrum includes aspects from purification of fresh media of fermentations, separation of biopharmaceuticals, polishing of protein products [1-3]. Purification and separation using microfiltration and ultrafiltrations are being studied intensely due to the versatile nature of the research and good accessibility in research facilities. The past decade has seen a vast growth in biopharmaceutical industry and presently in developing

countries such efforts are also being made. The macromolecule productsfrom bio-industry [4] received continuous attention in membrane filtration research area.

Meanwhile, nanotechnology has been incorporated into biomedication and smart materials. [5-7] The special properties of nano-scale material are generally new and interesting in material science. Among the bio-relevant properties are the large specific area, [8] controllable size, [9-10] well-defined structure, [11] facility of functionalization [12] and spectrophotometic availability, [13] towards which efforts from various research area has been put in. Nano-scale smart materials such as microgels, [14-15] nanocomposites and modified nanocrystals [16-17]. have been successfully developed. Promising results include, a complete regression of tumor in vivo, [18] targeted *in vivo* detection [19], biodegradable materials [20], biocompatible modification of proteins PEG [21]. However, purification is overlooked as most attention goes into new functionalities, advanced preparation techniques, combinatorial applications and bleeding edge explorations. A matching effort should be made since there is a great opportunity for all kinds of applications out of the competence from membrane filtration. The processing of these high valued products could lead to more attention of funding to help the field develop and lower the cost in making the latest bionanotechnologies accessible to a larger group of recipients [22-24].

Membrane techniques have utility in not only the processing of biomedical products but also the studying of their properties. Although much research has been done conventionally with the focus on the properties of membrane, sometimes it is the property of the solute that makes a specific topic interesting. Supramolecular interactions may

have an impact on the mass transfer in membrane filtration processes as suggested by the following researches. For example, poly(N-isopropylacrylamide) microgels in pressurized feed are reported to be deformable in solution [25] so extra consideration is needed when such material is used in microfiltration and ultrafiltration processes. Moreover, there is research on vesicular material undergoing reconstruction as a vesicle goes through the membrane [26]. Aside from the soft matter mentioned above, biological macromolecules, such as DNA and protein, having strong intramolecular interactions are also reported to be responsive to the environmental condition where they are being filtered [27]. External modification of a protein will also change its surface properties and put an impact to the filtration process. [28] Membrane technology itself is involve with nanotechnology, biomedication, and analysis and should be accepted as more than a way of preparation samples. Along with all the traditional establishments of membrane fabrication and other applications of membranes, membranes on biomedical and analytical applications are prospected to have a prospering future.

1.1.2 Considerations on analytical methods

The fast and accessible performance of membrane also allows further development of new analytical tools. Protein-drug binding is an important research area of pharmacokinetics, [29] since the extent binding to serum albumin is a big factor influencing the performance of the drug. A widely accepted hypothesis is the “free drug” model, where it states the fraction of the drug dosage that is therapeutically relevant is the unbound drug in the system after the administration. Meanwhile the concentration of a specific drug at the region of action must exceed a certain value for the drug to be

effective. The analysis for the unbound drug usually is done by a separation followed by one step of detection [30]. There have been already many mature systems with sophisticated instrumentation [31-33].

Many analytical tools involve online detection of the analyte. While these means produce reliable and in-depth information, sample preparation and operator intervention are mostly inevitable. Usually reloading of another sample, washing of the media, employing a new ELISA plate and re-stabilizing the instrument are commonly required. This not only result in increased time of conduct, energy consumption but also adds on to the cost and time spent.

There are scenarios in which the time for testing the drug-protein interaction is a more apparent issue. Ideally a system that allows real time loading of samples can solve the time offset brought by the traditional means of detection mentioned above. The requirements of the equipment may not be as high as those with a high sensitivity and accuracy. By introducing a tangential flow system, samples are consecutively introduced to the separation unit and the unit itself is regenerated by extended buffer washing. During the course of separation, when the signal reaches the baseline again, the system is ready for another sample without manual reassembling of any parts. The sample injection be automated, the outcome of the system can be unattended self-operated, giving the possibility of testing a large number of samples with a single deployment. As the flow is simple and robust in nature, the system is inherently stable in operation.

The sensor for quantification of the analyte of interest can be adapted from capable standalone UV detectors or the ones from HPLC systems. The readily available software

on computers further reduces the first time investment and gives the solution a wider applicable range. Especially in field operations or demonstrational sessions where a quick test can be just enough or the principle of the operation is of importance, such systems can be viable alternatives. If the sophisticated application is favourable, this prototype can also be integrated with microfluidics and existing high-end analytical measurements for better substantiated performances.

1.1.3 Fouling and concentration polarization

A few noteworthy disadvantages accompany the aforementioned technical superiorities of membrane filtration. Fouling and concentration polarization are widely happening in most of the ultrafiltration and some of the microfiltration processes. In the case of membrane fouling, the membrane is blocked by particles or solutes from the medium that is being filtered [34]. Fouling could result in decrease of permeability, increased pressure, and eventually render the membrane unsuitable for use [35]. There has been wide interest in fouling of membrane for its mechanisms and preventions within the field of membrane science [36-37]. Concentration polarization is when the concentration of a solute is significantly higher at the wall of the membrane than that from the bulk of the solution, and decreases with a boundary layer adjacent to the membrane [38] during a filtration process. It is known for impacts on the performance of mainly ultrafiltrations, such as pressure build-ups, reduced permeability by osmotic effect across the membrane [39]. Traditionally, such effects are considered to exert negative influences on the process and thus avoided wherever possible [40-41]. Several fouling mechanisms proposed the solute is entrapped in either the membrane surface or inside the pores [42]. The solute

distribution in concentration polarization has also been modeled. The size of the solute is known to be a major factor influencing the extent of such polarizations [43].

However, if decoupled from the their effects on the final results in the researches where parameters related to productivity or performance are of concern, these perceived short comings are in nature a matter of solute distribution. As in this thesis the membranes were used primarily for its barrier effect. Another perspective was taken to explore the effects of fouling and concentration polarization. For fouling, if the fouling agent is detectable, or another detectable substance that is incompatible with the fouling agent could be introduced in the filtration, membranes could be used as a substrate for fine pattern transferring or visualizing. As in microfiltration and ultrafiltration the pore sizes are generally under one micrometer, the "pixel" size in this case, if such application is feasible, is well above the points per inch (PPI) values of all mainstream digital-optical imaging devices. Thus if the solutes can be visualized or contrasted, with appropriate magnification technique, fine patterns could be extracted with high fidelity and precision. In other cases, concentration polarization, on account of being one case of solute redistribution, can show some value in developing analytical tools and be better illustrated to reveal more information of the filtration process.

1.2 Thesis outline

1.2.1 Chapter two – microfiltration of microgels

In chapter two, the investigation of purification of Poly(N-isopropyacrylamide) based microgels were combined with the observation of the process as well as the new findings about the basic properties of microgels in physiological conditions. In some preliminary experiments where the design was to achieve total retention of microgels to look for possible purification and bioseparation applications, the counter-intuitive massive transmission of microgels through membranes with porosities significantly smaller than the particle size of microgels spurred the following detailed research. The transmissions of microgels were inherently stimuli-responsive due to the same behaviours of microgel particles. Apart from confirming such properties, a hypothesis was made based on the considerations of the nature of the structure of the microgel particles that these particles are extensively flexible in their aqueous solutions, and supported by raising the flux of microfiltration over a critical value such that substantially increased transmissions were observed. The condition for total retention was found by decreasing the molecular weight cut-off of the membranes used. Samples of microgel synthesis crude mixture were purified according to the findings mentioned before and the performance was promising. Some implications on *in vivo* applications of microgels and bioseparations of macromolecule products of biological origin are also discussed.

1.2.2 Chapter three – an analytical method for analyte-ligand binding

In chapter three, an analytical method to study protein-drug binding was developed and validated. As the same in the previous chapter, membrane was used as barriers. 10k ultrafiltration membrane was used to retain the protein molecules and letting free drug molecules pass. Tangential flow filtration (TFF) was employed for ease of cleaning and reusability of a single assembly. Pulsed injection was used as a means of generating comparable results between different runs using identical parameters. The set-up was derived from an existing HPLC system by replacing the column with a TFF membrane module for interoperability. The module was custom-built and an augmentation was implemented to eliminate bubbles formation for the UV sensor. Fine changes of flow rates on the feed and permeate line were made. Some comments regarding the performance of the system were also made. Data for the binding of a model combination of aspirin and bovine serum albumin (BSA) were obtained and interpretations to show trends made.

1.2.3 Chapter four – analytical applications of membranes

In the following chapter, some miscellaneous findings of unorthodox usage of membrane as barriers were compiled. While traditionally considered negative side effects in microfiltration and ultrafiltration, fouling and concentration polarization carry information. In the nature of fouling, the solute is immobilized locally to the filtering medium. Body grease, the lipid substance present on the surface of skin is able to foul a certain number of membranes. Cellulose acetate and polyethersulfone microfiltration

membranes were used to capture fingerprints by absorbing the lipids on a fingertip. Aqueous deep blue-dyed latex suspension was then used to selectively foul the rest of the area. It could be an alternative way of storing biometric information and show superiority in some aspects. The same latex beads were also used in a tangential flow system where the membrane in the module covered a supporting with a small opening in the middle. Instead of getting a square shape, a circular accumulation profile was observed with a blank area in the middle concentric to the opening on the support. A hypothesis was made the membrane and the flow in the vicinity of the entrance of the square opening will adapt into a condition such that the net flow through the membrane is maximized to reduce the resistance of flow. For verification, the module was further tightened, forcing the membrane to sit flush and seal against the support, leaving only the opening accessible to the flow. An experiment with the same parameter settings achieved accumulation in identical shape to the opening.

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2. Microfiltration of microgels

2.1 Abstract

Poly(N-isopropylacrylamide) (pNIPAM) microgels have been studied for their potential use as drug delivery vehicles in the biomedical field. However, before they can be used for such application, the have to be purified to remove unreacted monomer, linear polymers, macrogel debris and other impurities. Conventionally, microgels are purified by repeated dialysis against fresh water, which is a diffusion driven process that requires days to complete, or via ultracentrifugation that requires intensive operator attention to repeatedly remove supernatant and redisperse microgels over multiple centrifugation cycles. Microfiltration and ultrafiltration are pressure-driven, convection based techniques which offer the potential for fast purification of microgels. However, due to the potential for deformation of microgel structure and the responsiveness of pNIPAM-based microgels to environmental conditions such as pH and salt concentration, separation and purification by membrane filtration is challenging. Model microgels were covalently tagged with Rhodamine B to enhance detectability and hence measurability. The effect of salt in the feed on the transmission of microgels was investigated in both constant ionic strength and changing ionic strength environments. Microfiltration and ultrafiltration membranes with different pore sizes were used to examine the deformability of microgels. The effect of microgel stiffness was explored by synthesizing microgels with different

crosslinking densities. The stimuli-responsive behavior of a microgel was found to have a significant influence on its filterability.

2.2 Introduction

Microgels are spherical cross-linked polymer networks with a size on the micron to submicron scale. [1],[2] Microgels based on poly(N-isopropylacrylamide) (NIPAM) are thermo-responsive and can undergo a volume phase transition at ~32˚C in aqueous suspension, analogous to the lower critical solution temperature (LCST) behaviour of linear pNIPAM.[3] Given the proximity of this "smart" transition to physiological temperature, a variety of biomedical applications of microgels have been proposed, including their use as drug delivery vehicles, biosensors, and purification/separation matrices.[4]. However, for safe use as biomedical materials, microgel particles need to be completely purified to remove unreacted monomer, crosslinker, initiator residues, oligomers and linear polymer chain fragments since such substances can either be toxic in vivo or interfere with the desired experimental phenomenon [5]. The convention for post-synthesis purification is typically repeated dialysis cycles against fresh water for at least six cycles of six hours each. [6] Although dialysis offers acceptable performance in terms of product purity, the nature of a dialysis as a diffusion-driven process suggests there could always be some small molecule impurity in equilibrium left behind by conventional dialysis purification.[7],[8] In some cases, traces of impurity could cause severe reactions in vivo [9], demanding a higher level of microgel purity. Alternately, repeated cycles of ultracentrifugation have been performed in which the supernatant is removed, replaced with distilled deionized water, and the microgels are redispersed for another

centrifugation cycle, typically continuing until the conductivity of the supernatant is <5μS/cm. [10] Again, though, the purity of the product depends on an equilibrium between the undesired materials in the (pelletized) gel phase and the supernatant phase, demanding multiple purification cycles with significant operator intervention at each step that is not amenable to scaled-up processing.

Membrane filtration is a pressure-driven process in which convection is the major mode of mass transfer. Compared to dialysis, membrane filtration offers a higher rate of mass transfer, uses less water, and allows the practical possibility of complete removal of small-molecule impurities. Membrane filtration has been widely applied in the research of separation, purification, and polishing of biopharmaceuticals. To the best of our knowledge there is no work done on using microfiltration or ultrafiltration as the purification approach for microgels. Therefore, it is beneficial to explore the potential application of ultra/microfiltration techniques for microgel purification that would result in higher purity products, are cost-effective and allow for shorter processing times.

The unique properties of microgels pose both challenges and opportunities for purification via filtration-based processes. Microgels are not rigid spheres under any environmental condition and undergo changes in both size and elasticity as a function of temperature, ionic strength, and (in many cases) pH.[11] Previous studies have shown that microgels can significantly deform upon shear, including resistive pulses during the translocation of microgel particles larger than a nanopore electrode [12], [13] and the deformation and dehydration of PNIPAM-based microgels through glass membranes [14]. Shearing of soft microgel pastes cause changes in the microgel microstructure [15] and

thus colloidal stability [16], offering the potential of shear-induced aggregation. In addition, PNIPAM microgels are hard to quantify or even detect at very dilute concentrations, making tracking of the success of the purification protocol challenging.

The deformability of microgels brings particular challenges to the design of the purification process. The purification of linear polymers or proteins generally requires a compatible molecular weight cut-off (MWCO) for the target polymer/protein and an appropriate buffer to carry out the process [17]. However, it is hypothesized the MWCO for practical purification of microgel particles is subject to the extent these particles will deform under those specific experimental conditions. Moreover, in some biological objectives such as protein fractionation, not only the MWCO but also the surface properties of the membrane [18], [19] and the buffer solution need to be carefully selected; in the latter case, a dynamically-changing buffer solution is sometimes required to separate materials with different hydrophobicities[20]. As pNIPAM possesses a VPTT that relies on temperature-dependent interactions between the microgel and water, the swelling of PNIPAM microgel particles will vary depending on the type and concentration of ions present in the suspension during their filtration purification [21]. As a result, several constant and dynamic concentrations of NaCl solutions were used to probe the optimal methodology for purifying microgels. As well, the hydrodynamic sizes of each of the microgels in feed solutions containing a range of NaCl concentrations were tested. To enhance the detectability of low concentrations of microgels in the permeates, the microgel samples received contains microgels copolymerized with a methacrylate comonomer bound with fluorescent Rhodamine B. Finally, based on the information

collected above, a sample purification of the reaction mixture after microgel synthesis.

2.3 Experimental

2.3.1 Materials

All water used in the synthesis and characterization steps was of Millipore Milli-Q grade unless otherwise stated. Sodium chloride (NaCl) was purchased from BioShop Canada Incorportated, dissolved in water to make a range of salt concentrations (0mM to 2M NaCl solutions), and microfiltered and degassed prior to use. A 0.2 micron pore size Isopore membrane was purchased from Millipore. Cellulose acetate microfiltration membrane with 0.8 micron pore size was purchased from Sartorious. The 3k, 10k and 50k molecular weight cut off (MWCO) ultrafiltration membranes were purchased from Pall Inc. All membranes were used as received. Microgel samples were provided in freeze-dried powder form and reconstructed into solutions using water.

2.3.2 Microfiltration and ultrafiltration of microgels

Microgels were redissolved in deionized water at a concentration of 2.4 mg/mL. A custom stirred-cell membrane module with 1.2 mL volume was used to house the filtration membrane, as shown in Fig. 2.1 (A). In this section, one 0.22 μm Isopore membrane was supported by two cellulose acetate microfiltration membranes for mechanical strength. A sample loop of 50μL was used on the injector upstream to the membrane module. The feed was pumped in using HiLoadP-50 pump (GE Healthcare, product code: 19-1992-01) at a constant flow rate of 0.1 mL/min. A 0.2 mL sample of the

permeate was collected every 120s into a single well of a 96 black well plate, later used to measure residual microgel concentrations via fluorescence. Ultrafiltration membranes were installed without supporting membranes on account of their mechanical strength. To illustrate the absence of particle – pore interactions, a control was carried out using an identical set-up and specifications as the deionized water experiment above, with a punctured hole in the membrane stacks. For higher flow rates, the time was recalculated against the relative speed of the 0.1 mL/min experiment.

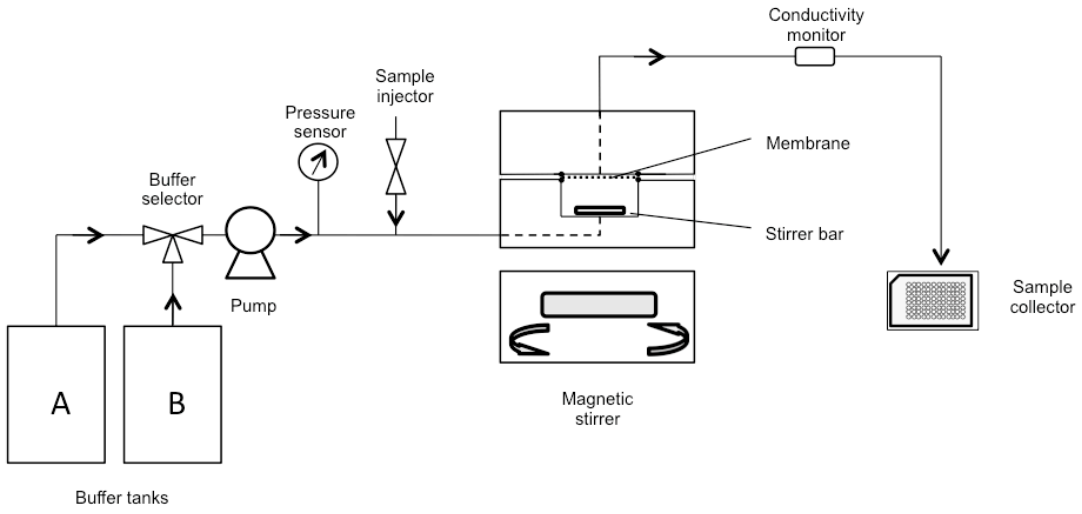

Figure 2.1 (A) Schematic of set-up of microfiltration and ultrafiltration of microgels

2.3.3 Fluorescence detection

Collected permeates were measured for fluorescence (i.e. residual microgel) using Black Cliniplates (Thermo Scientific) and a Fluoroskan Ascent FL fluorimeter (Thermo

Electron Corporation), using an excitation wavelength of 544nm and an emission wavelength of 590nm. Samples were measured immediately following the filtration experiments. The values reported represent the average value of five replicate measurements, with the experimental uncertainties representing the standard deviation of the replicate measurements.

2.3.4 Ultrafiltration of microgel reaction mixture

The purification set up used similar equipment and is shown in Fig. 2.1 (B). For the consideration of reducing human presence to monitor the entire process over the possible long periods, suction mode was chosen, as there is no over-pressure situation as the maximum transmembrane pressure in this case always lower than one bar. The crude mixture after the synthetic reaction of microgels was provided used in the following purification without further treatment. Ultrafiltration membrane of 10k MWCO was used to carry out the pilot purification. A stirred cell with 100 mL capacity was employed to house the membrane. Gentle stirring was kept constant throughout the purification process. The sole opening of the well sealed membrane module on the top of the retentate compartment was connected and dipped into a covered flask. Fresh Milli-Q water from the flask was replenished by percolation driven by atmosphere as the permeation caused decrease in the volume of the retentate. Samples were taken at different time intervals and analyzed on NanoDrop 2000 for UV absorbance at 195 nm to evaluate the impurity level. Performance was analyzed and compared with model purification data supplied by collaborators.

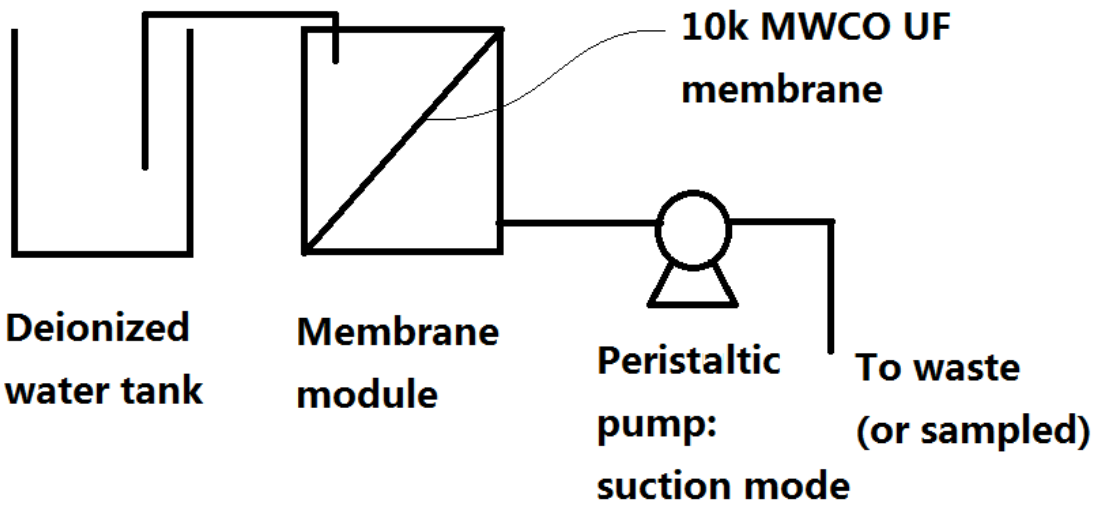

Figure 2.1 (B) Schematic for purification microgels using ultrafiltration

2.4 Results and Discussion

Considering the Isopore membranes used for this work have etched tunnels with very little variance in their individual pore size, the microgel-membrane interaction will contribute to the resistance of the transmission to a large extent. Thus, if the sizes of the particles are comparable to the pore size, subtle changes in the particles' physical structure could significantly change how the microgels interact with the pores and therefore significantly change the amount of transmitted microgel particles. The transmission of microgels through an Isopore membrane is highly responsive to the NaCl concentration in the feed, as shown in Figs. 2.2 (A) (by concentration) and (B) (by

cumulative mass transported). As the concentration of NaCl in the feed was increased from 0mM (deionized water) to 600mM, the microgel transmissions through 0.22 μm Isopore membranes decreased. When the feed was 2M NaCl, the transmission became significantly less than those observed at lower salt concentrations. The same trend is reflected via measurement of the retentates of the experiments (Fig. 2.2 (C)), which indicates that the amount of microgel held in the membrane module after each experiment increases as a function of salt concentration. The transmission behaviour in the control experiment using deionized water and a set of punctured membrane in the same specification (100% transmission) clearly shows the level of transmission as well as the cumulative mass are both significantly higher than those subject to a well-performing membrane stack (Figs. 2.2 (A) and (B)).

Microgels are colloidally stabilized both by steric interactions (at temperatures below the VPTT) and electrostatic repulsion driven by the presence of anionic sulfate residues on the microgel surface (from the persulfate initiator decomposition). Increasing the ionic strength thus causes microgels to first shrink and subsequently aggregate into macroscopic precipitates, reducing the probability of single particle slipping through the pores in the membrane. This aggregation effect explains the mild decrease of transmission shown in Fig. 2.2 (A) and the decreasing cumulative mass transported versus time in Fig. 2.2 (B) as a function of salt concentration.

If the proposed mechanism should be true, dynamic NaCl concentrations will cause the profiles of microgel transmission to change as a function of the environment. For example, if the NaCl concentration is ramped from 2M to 0M (deionized water) as the filtration

carries on, we would expect a second peak in the transmission versus time curve at the

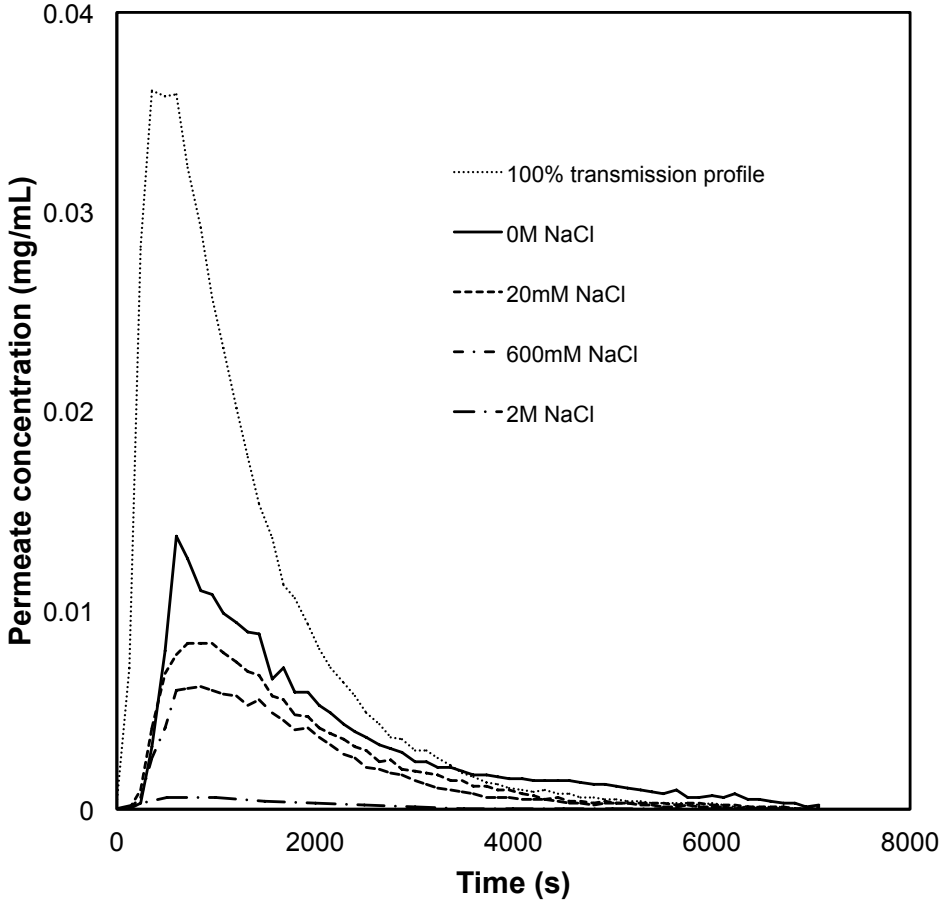

Figure 2.2 (A) Transmission profiles for an injection of 2.4 mg/mL microgels sample in deionized water (0M), 20mM, 600mM and 2M NaCl feeds. The 100% transmission profile was the yield when the membrane stack was punctured.

critical aggregation concentration of salt for the microgel being investigated. Results in Fig. 2.3 displayed the anticipated phenomenon. Due to the decrease in ionic strength the aggregated microgel particles re-dispersed and subsequently were transmitted through the membrane. The shapes of the transmissions were analogous to normal chromatographic

peaks, suggested that the redispersion process is fast compared to the rate of change in NaCl concentration and redispersion is a single-stage event applicable to the entire population of particles. This observed facile and complete reversibility of microgel aggregation is consistent with physicochemical studies of this phenomenon and could be

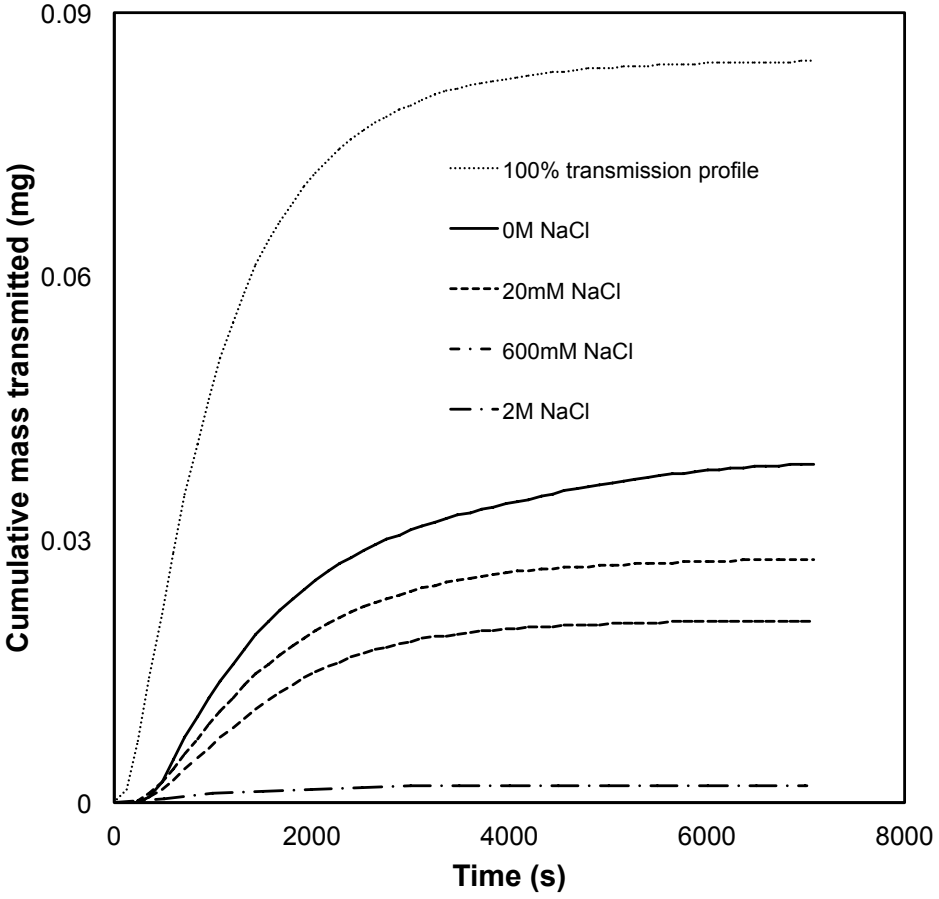

Figure 2.2 (B) Cumulative mass of the respective conditions.

taken into consideration when designing in vivo drug delivery vehicles using microgel based materials.

As an extreme case with a feed change from 2M NaCl to deionized water, the last

experiment shows only the aggregation-related dynamic transmission. Moreover, the ionic strength, in this case the concentration of NaCl, can stimulate the change in transmission profiles by moving in and out of the ionic strength range in which aggregation happens. Fig. 2.4 shows the transmission of microgels in an experiment in which the feed was started at 2M, adjusted to 600mM NaCl for 72 minutes, and then subsequently changed to deionized water. The transmission profile reflected this step change in ionic strength by exhibiting three individual transmission bursts. Therefore, it

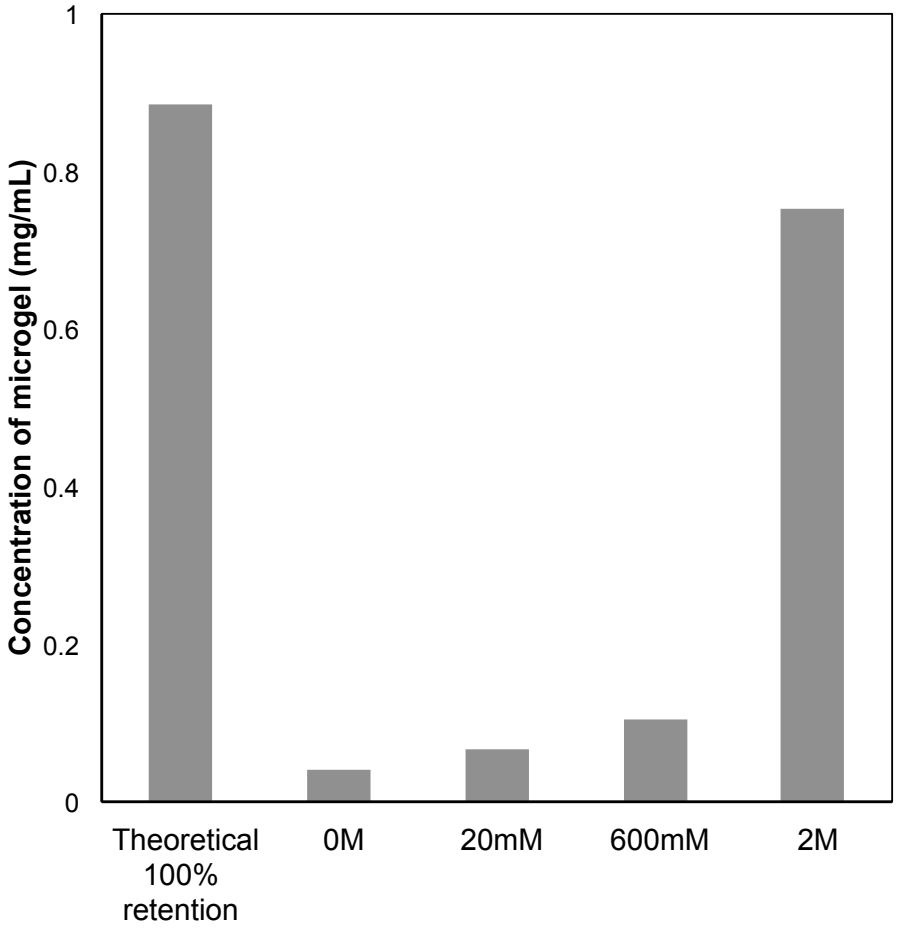

Figure 2.2 (C) Concentrations of microgels in retentates in the respective experiments.

can be concluded the state of the microgel particles are essentially different in 600mM NaCl and deionized water, with a significant decrease in the resistance to transmission at each ionic strength step providing pulsed boosts of the concentration of microgel in the permeates.

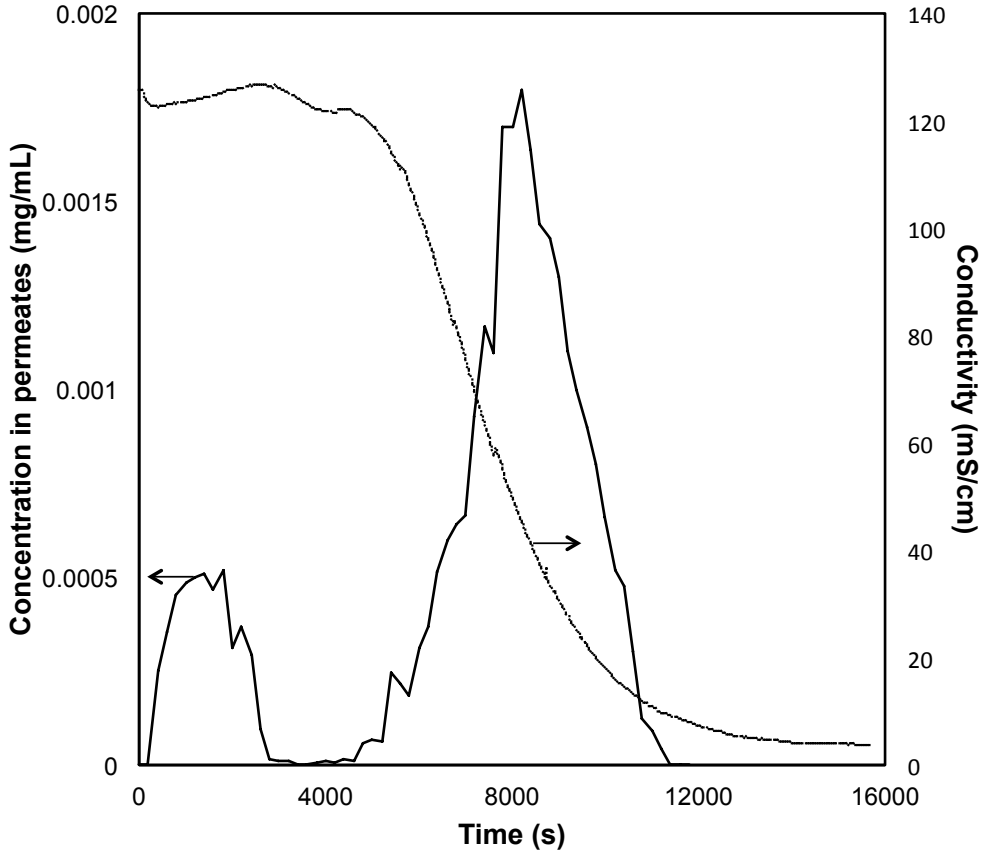

Figure 2.3 Dynamic transmission profile for with a step change of feed from 2M NaCl to deionized water. A second round of release was observed.

As another consequence of this salt-induced change in microgel aggregation state, we

would expect the transmission to be shut down if the NaCl concentration keeps increasing and exceeds a certain critical value.

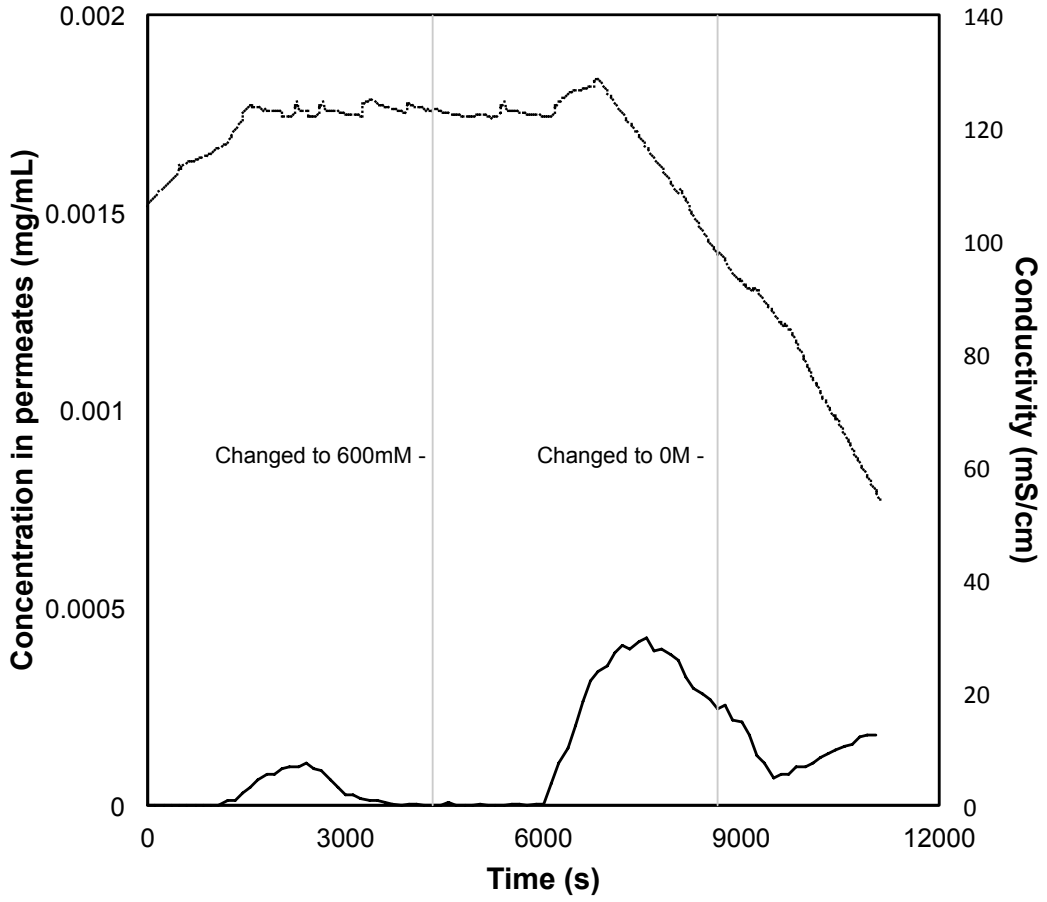

Figure 2.4 Dynamic transmission profile with two step changes of feed from 2M NaCl to 600mM then deionized water. Note the third release developing from 9500s

Fig. 2.5 shows the results of one such experiment in which the feed was changed from deionized water to 2M NaCl over time. The transmission behavior demonstrates a quick and clean stop in microgel transmission as the NaCl concentration is increased. Because the feed was changed to 2M directly, the increase of NaCl concentration in the membrane

module was quick. Furthermore, the transition of microgel particles from dispersed condition to aggregation is known to be quite rapid. [22] As a result, the favorable conditions for microgel particle transmission were eliminated within a short time span due to the rapid change in ionic strength and apparent transmission was terminated. This result also further substantiates previous observations that aggregation changes are faster than the rate of the conductivity change.

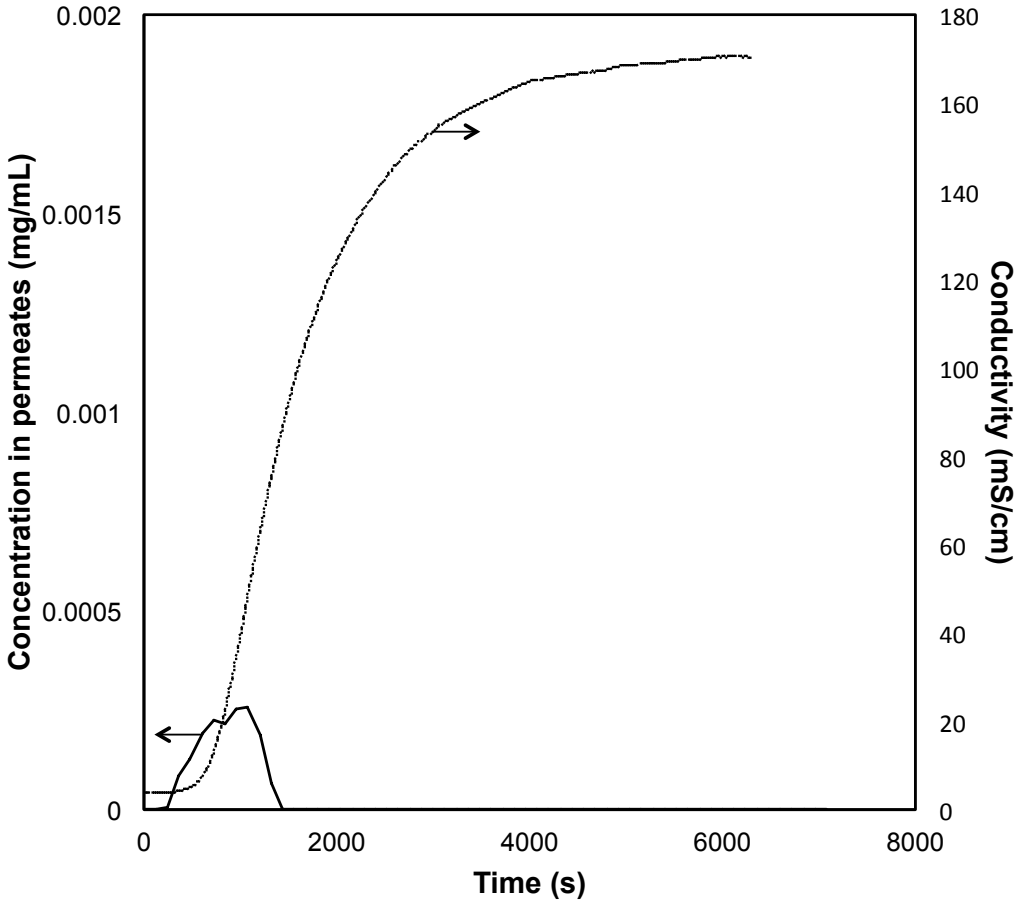

Figure 2.5 Dynamic transmission profile with a step change of feed from deionized water to 2M NaCl. A shut-down effect to the transmission was observed.

While it generally holds that a 50k ultrafiltration membrane will retain protein molecules such as IgG (150 kDa, smaller than 25 nm in solution), microgel particles need extra attention when it comes to the feasibility and utility of their purification. While protein molecules can be considered non-compressible in many scenarios due to secondary structure formations, such as S-S bond and hydrogen bond, microgel particles are extensively deformable under shear stress[23]. As such, traditionally cited molecular weight cutoffs many not apply to microgel particles.

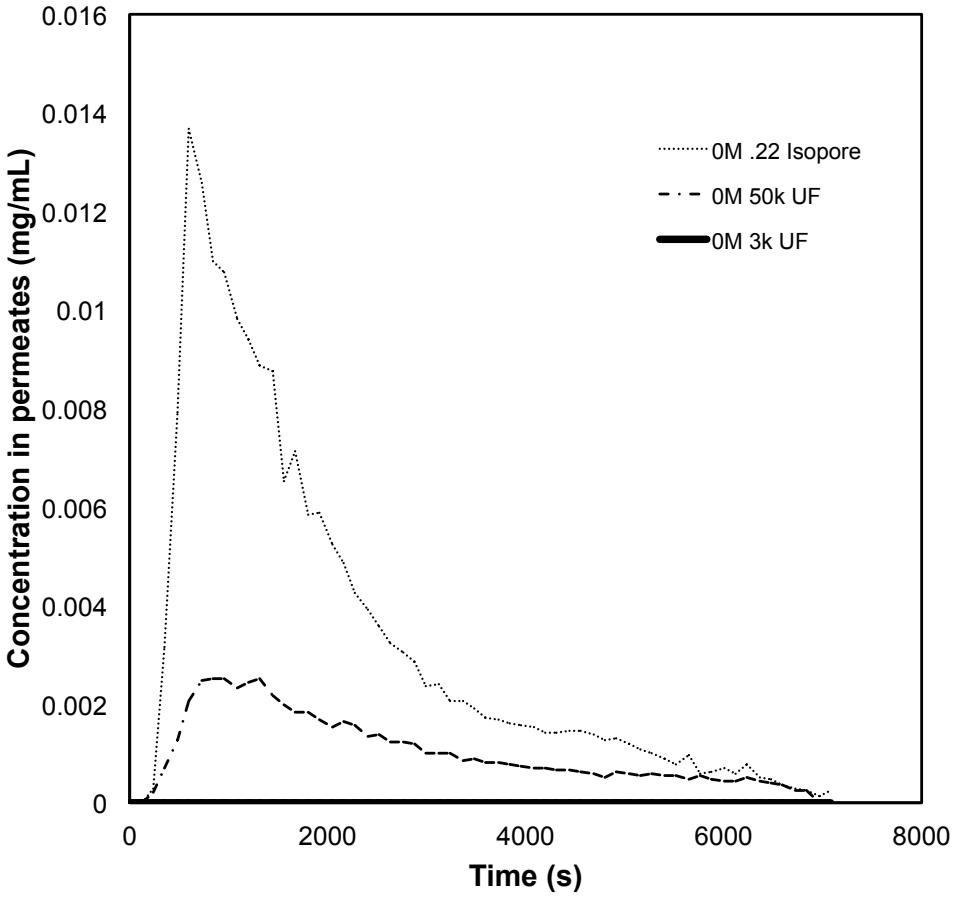

Figure 2.6 Transmission behaviors of the same microgel through .22 Isopore membrane, ultrafiltration membranes of 50k and 3k MWCO.

Results for filtration using 3k and 50k MWCO ultrafiltration membranes and a 0.22 μm Isopore microfiltration membrane using deionized water as feed are shown in Fig. 2.6. Even though the 50k membrane is sufficient for total retention of IgG, it is still partially permeable to the microgel, although the magnitude of the transmission is lower than that observed for the microfiltration membrane. Since the hydrated diameters of the microgel particles are in the hundreds of nanometers, one magnitude larger in size than IgG molecules, this result is only possible if the microgels are forced through the pores via microgel deformation to fit the etched tunnels of the Isopore membrane. Of note, the 3k UF membrane showed a series of explicit zero readings of microgel transmission, further reinforcing that the detected fluorescence is not attributable to the presence of labeled PNIPAM oligomers or residual monomer but rather the microgel particles themselves (i.e. the initial dialysis step performed to clean the microgels prior to the filtration experiments was effective in removing small molecules bearing the fluorescence tag). Therefore it is advisable to purify microgels using a UF membrane with very low MWCO instead of choosing an MF process based on the hydrodynamic size of the microgel particles being filtered. The results also clearly indicate that deformability of microgels must also be strongly considered in applications of microgels in which semi-permeable barrier is present.

In attempt to an explanation of the salt responsive transmissions, we propose the extensive flexibility hypothesis. As they are extensively hydrated crosslinked network these microgels can be well considered a semi-fluid within the spherical matrix of one single microgel particle, to a degree that the flexibility change put in impact on the

transmission behaviours. Figs. 2.7 (A) and (B) shows the transmissions at fluxes of 8.83, 17.7, 26.5, 35.3, 44.2 × 10-6 m/s. A clear increase in transmission due to flexibility effect can be observed in the two experiments with higher fluxes as opposed to the ones with lower fluxes, where the transmitted mass were largely in the same ballpark with each other. Put together, the salt responsiveness exerted an effective change in the flexibility of the microgels by altering the solution condition. As a result the transmission profiles were changed.

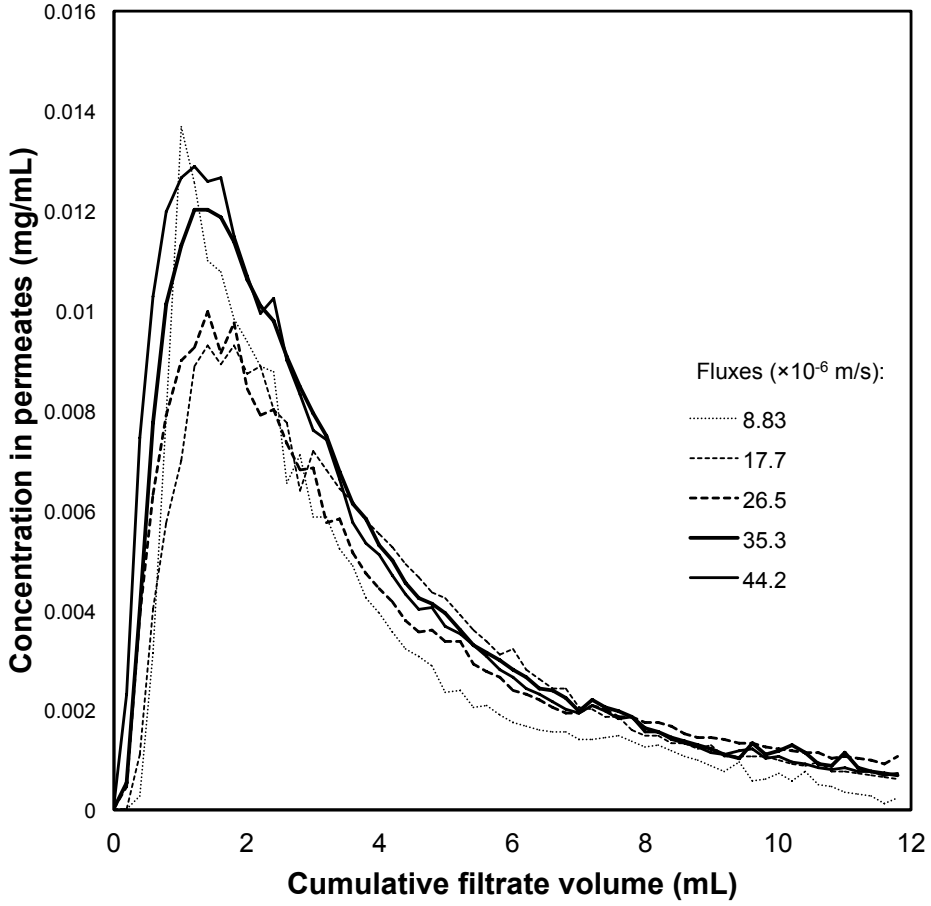

Figure 2.7 (A) Transmission profiles of microgels of different flow rate used.

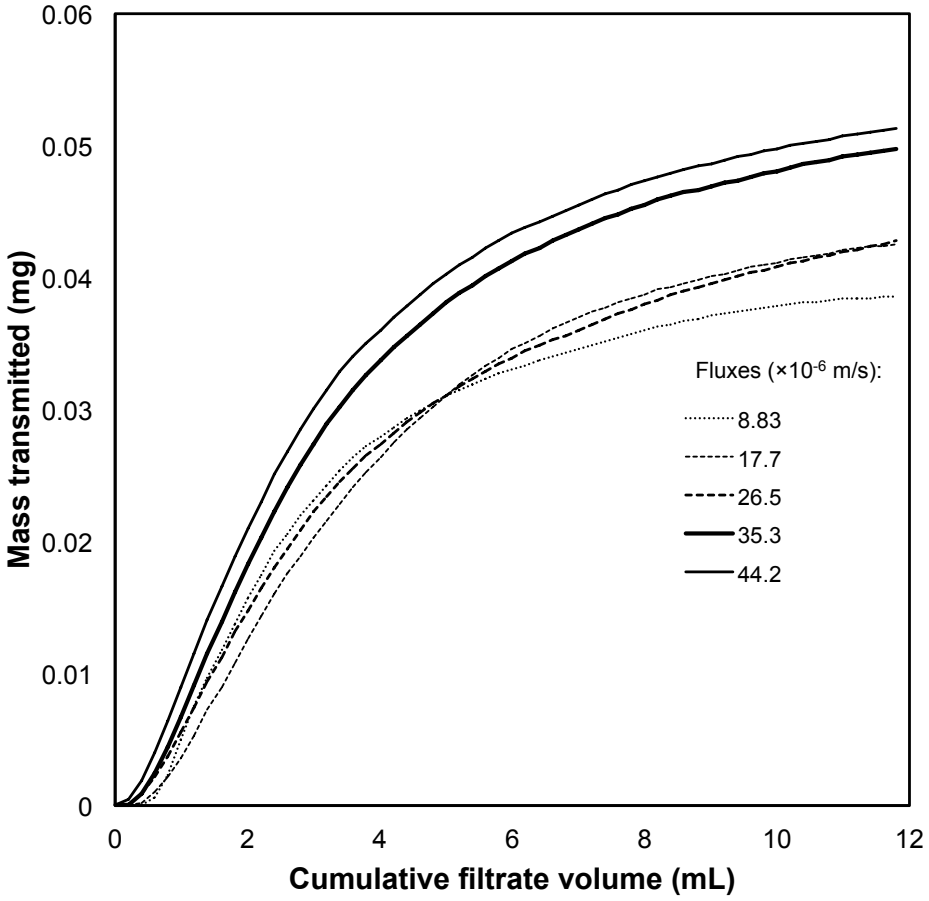

Figure 2.7 (B) Cumulative mass of the respective trasmissions.

Stemmed from the investigation and suggestion above about what not to do in membrane filtration of microgel, an experimental purification was done and the impurity level recorded during the course of ultrafiltration in Fig. 2.8. The organic impurity of unreacted monomer and crosslinker both have carbonyl groups that absorb UV light at 195 nm. The respective absorbance was used as a means to quantify the impurity removal rate. Samples were taken at varying intervals, starting at 10 min./sample then slowly decreased to 20 min and hours in later stage. Although the data set is preliminary in nature, it still

reveals the trend and high time efficiency in removal of small molecules compared to days of dialysis. As part of the goal to find a less operation intensive alternative to dialysis in microgel preparation, 10k ultrafiltration may be a viable choice.

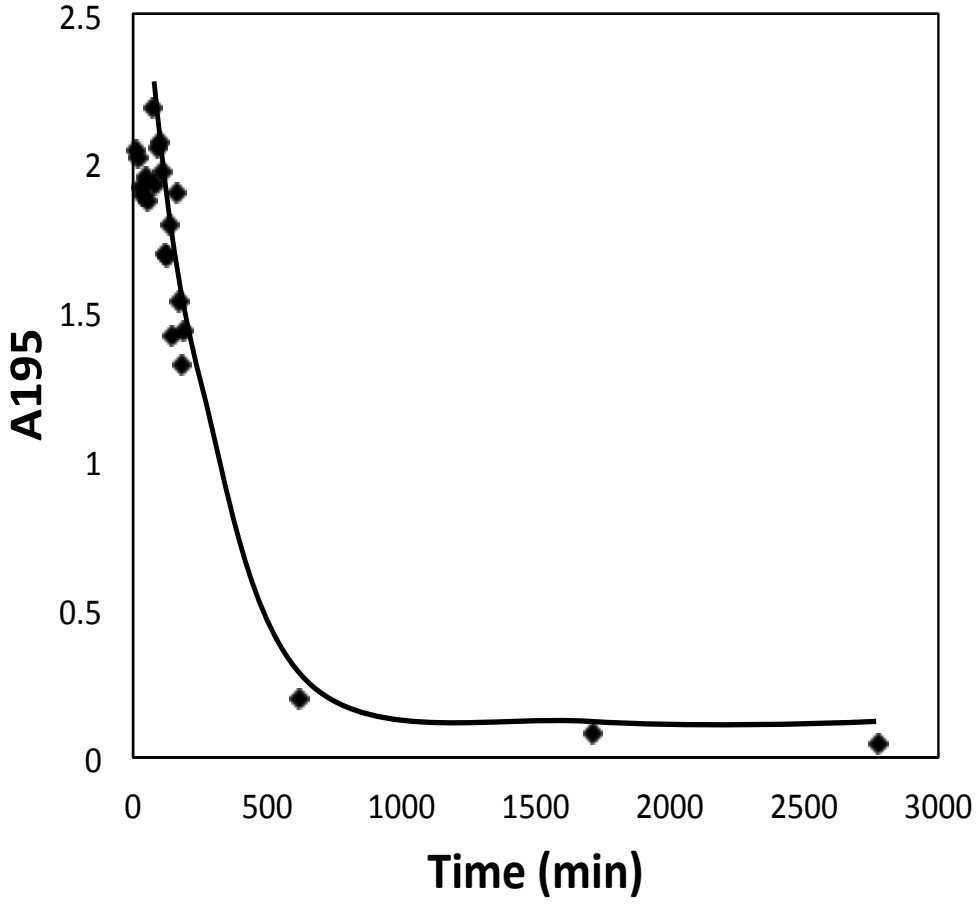

Figure 2.8 Impurity removal profile

2.5 Conclusions

Microfiltration of PNIPAM microgel was investigated as an alternative to conventional dialysis in pursuit of identifying a faster and more scalable microgel purification process.

While such a separation has been demonstrated to be feasible, the molecular weight cut-off of the target membrane cannot be decided based on the hydrodynamic size of the microgels, unlike with proteins. A size ratio of particle-pore of up to two orders of magnitude was observed to maintain some degree of transmission through the membrane, according to the ability of microgels to deform under shear. As a consequence, in both purification and actual applications, the morphological changes in microgel particles as a function of changes in the microgel environment need to be accounted for wherever possible.

The ionic strength was confirmed to exert a dominant influence to microgels' transmission behaviors, with as little as 20mM NaCl needed to cause the amount of transmitted microgel to decrease two-fold. The transmission behaviors only underwent benign changes in isocratic experiments within the range of 20mM to 600mM NaCl, over which range the microgels remained dispersed; however, further increasing the salt concentration up to 2M NaCl induced microgel flocculation into macroscopic flocs that were significantly larger than the microfiltration membrane pores resulting in an almost extinct transmission. Experiments with dynamic NaCl concentrations demonstrated that the transmission of microgel is rapidly responsive to changes in the ionic strength in the feed solution. Salt concentrations higher than 600mM would terminate the transmission while the transmission is enhanced by feeds with less NaCl added.

Due to the flexibility, projected retention behaviours in vivo can be different from the real case. Some biological porous media may not retain the microgels thus there is a loss of the useful material if the prediction is only based on size. On the contrary, the excretion

of microgels might also be possible if, again, by size, it is not originally possible. The effective flexibility also changes with salt, so the tonicity of targeted in vivo application area should also receive a considerable discretion. However, quantification of such effects should require further research.

The alternative of purification of microgels on a lab scale is found to use ultrafiltration of un-intuitively high retention standards, 10k ultrafiltration for microgels of hydrodynamic sizes in hundred of nanometers. Conventional frequent manual operations were eliminated. Instead, only one time collection of final product is needed and the system is fault-proof as the fresh medium was topped up into the filtration module by atmospheric pressure through suction, removing the risk of overflowing and high pressure build up.

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3. Study of protein-drug binding by pulsed tangential flow ultrafiltration

3.1 Abstract

In physiological conditions, the binding of small-molecule drugs to serum albumin is a key factor in pharmacokinetics. The free drug theory states that only the drug that is unbound is therapeutically relevant. To produce an effect the concentration of free drug must exceed a certain value at the location of effect. Many techniques are available and being studied in pursuit of accessibility, efficiency, sensitivity and precision, including mainly photometric and chromatographic approaches. However, conventional designs often involve heavy instrumentation. They are usually subject to intense manual operation as well as stringent on the requirements of the operator. Tangential flow is distinct in that the injected samples are self-cleaned quickly as the test progresses. Multiple samples can be analyzed in sequence with injections being the only necessary operation, which can also be automated. A pulsed tangential flow ultrafiltration based method with the potential in studying protein-drug interaction and macromolecule-ligand interactions in general is described. A model study of drug binding and non-binding to BSA is included as a proof of concept. The performance fine tuning were also explored in this paper.

3.2 Introduction

Drug binding is an important topic of research in the fields of molecular biology and pharmacokinetics. In these studies drug binding to serum is important to understand in that it affects the concentration of free drug as well as the disposition of the drug. The drugs' disposition and the amount of free drug is a key factor to consider when it comes to understanding how the drug will act as a medicine. It is hypothesized only the free drug is the actual portion of effect among the drug introduced [1]. Also, the drug is only effective when its concentration at its active site exceeds the concentration of therapeutic level [2]. Furthermore, the binding behaviour affects the disposition of the drug [3]. The binding data also sometimes give support to verifying the hypotheses on the drug binding, e.g. binding modes or equilibration modes.

Many techniques have been applied to this topic. Conventionally, the most abundant research attention goes to the chromatography and ultrafiltration based methods [4–7]. It was demonstrated that many detection techniques can be used after a separation process to extract the free drug from the incubate. Raman difference spectroscopy, [8] capillary electrophoresis, [9] NMR [3] and various approaches of mass spectrometry [10]. Whilst all the above mentioned methods supported good results only in terms of accuracy, sensitivity, it is not in all the cases that these performance factor are the most relevant.

Cost of test, level of instrumentation and speed are other important factors to take into consideration should these techniques be applicable for larger scale distribution in research facilities or hospitals. Complicated operation, heavy manual intervention, such as the use of probes, requiring specific machines to detect and not all probes work with all

drugs, higher learning curve, probes also require some extensive training to handle.

Non-specific binding has been a major challenge in various combinatory techniques where samples of ultrafiltration were collected and transferred to another means of detection, such as ultrafiltration-mass spectrometry [11] and ultrafiltration-HPLC [12]. In other cases immobilization of a probe as an aid of detection [13-14] is involved. The methods mentioned above involve high-voltage electric field, high flux magnetic field or complication of treatment of the analyte. While these are possible means to gain very detailed information of binding, not all applications need this much luxury. Hence, the proposed method in this study can still be justifiable due to its simple, low cost set-up and fast determination. Moreover, in conventional ultrafiltration utilizing a dead-end configuration, it has been suggested the equilibrium of drug adsorption to the membrane also contributes as a complication of the detection process [15].

Efforts have been made to minimize the technical barriers in the previous methods, such as the introduction of advanced calculation [7], putting the detection online with the separation step [13] and using single-step detection [16]. However, limitations can sometimes rise from these processes, for example, insufficient time for binding when the drug was passed through immobilized ligands [5].

In this chapter, a straightforward and robust design of tangential flow ultrafiltration is demonstrated. The system flow was driven by an HPLC pump. The incubate of drug and BSA was introduced in the feed line as a pulsed injection. The free drug was drawn by suction into the permeate and detected for UV absorbance. The feed line is reusable without dismantling the module. A flow meter downstream of the module was employed

for both the reading and augmentation of the permeate line to avoid bubble formation under suction. The drug-protein interaction is decoupled from the testing instruments allowing sufficient time for binding to equilibrate. The results can be obtained typically in less than 5 minutes with satisfactory resolution and acceptable reproducibility. Several small molecule drugs were tested as preliminary illustration of different modes of binding as well as concentration dependent binding behaviours. Meanwhile, the influence of the settings of the instruments on the performance of the whole system has been briefly explored.

3.3 Experimental

3.3.1 Material

Water used was produced from Milli-Q water unit with conductance of 18.2 MΩ and degassed before use. Drugs and BSA samples were purchased from Sigma-Aldrich (Catalogue No.) and used as received. PBS was purchased 10x concentrate and reconstructed when used. 10k ultrafiltration membrane was purchased from Pall and cut to sit in the module. The module was custom made with one acrylic top piece and one Delrin bottom part with a slit of mesh supporting the UF membrane. PEEK tubes and compatible fittings were used as the facility of connection.

3.3.2 Experimental set-up

This study employs a tangential flow ultrafiltration mechanism as the way of extracting from the injected sample unbound drug that is permeable to the membrane for UV

detection. The schematics of the set-up used for this study is shown in Figure 3.1. Briefly, 10k molecular weight cut off (MWCO) ultrafiltration (UF) membrane is sit in a tangential flow filtration (TFF) module as the selective barrier. The buffer was driven as the cross-flow from the buffer tank into the module by Waters 600E multisolvent delivery system as a pump with a sample injector. In the retentate line, a flow-meter was applied as a reading as well as restriction to augment for a steady permeate-flow. On the permeate side, a peristaltic pump was used in suction mode to send the permeate through the Waters 486 tunable absorbance detector. The voltage output of the sensor was then recorded in peaks with a computer for subsequent processing.

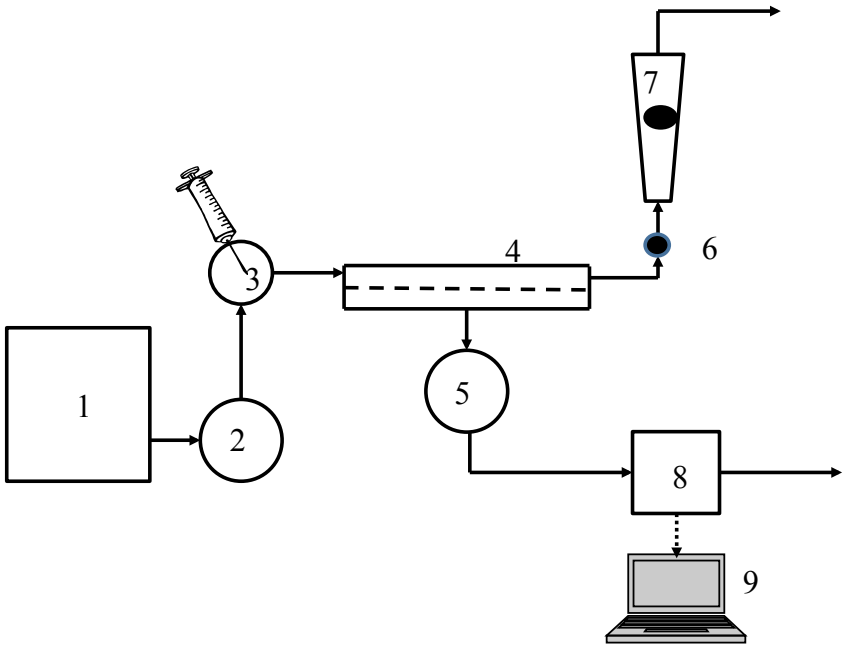

Figure 3.1 Schematic of experimental set-up: 1 Buffer tank, 2 cross-flow pump, 3 sample injector, 4 tangential flow membrane module, 5 permeate pump, 6 needle valve, 7 flow-meter, 8 UV detector, 9 computer

3.3.3 Experimental methods

Samples of aspirin were prepared in PBS. BSA powder was dissolved into PBS and kept in fridge until mixed under a 15s vortex with the drug samples. It was done such that the resultant composition is as described in the figure captions and wherever they are mentioned. The recorded peaks were for the extraction of area under curve (AUC) in area and peak heights in voltage as two parallel means of data processing to demonstrate the method's sensibility. To quantify, the calibration curves of these drugs were obtained to illustrate linearity between the signals and the actual concentrations.

3.4 Results and discussion

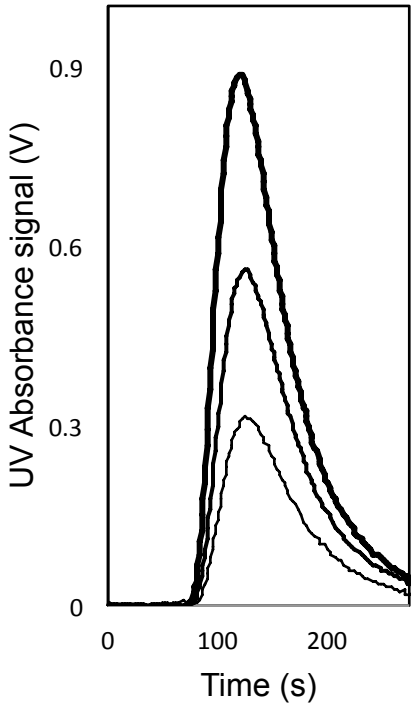

Figure 3.2 Overlay of free aspirin signals from incubates containing only PBS (heavy line), 4 mg/mL BSA (medium line) and 40 mg/mL (light line). Peaks only vary in height.

In Figure 3.2, the peaks of different free concentration of aspirin generally resemble each other, only varying by peak height. The peak heights and the areas under curve can be extracted for correlation of drug concentration and signal strength. In the presence of BSA, the drug binds and the amplitude of the peak reduces as a result of expected decrease in free drug concentration.

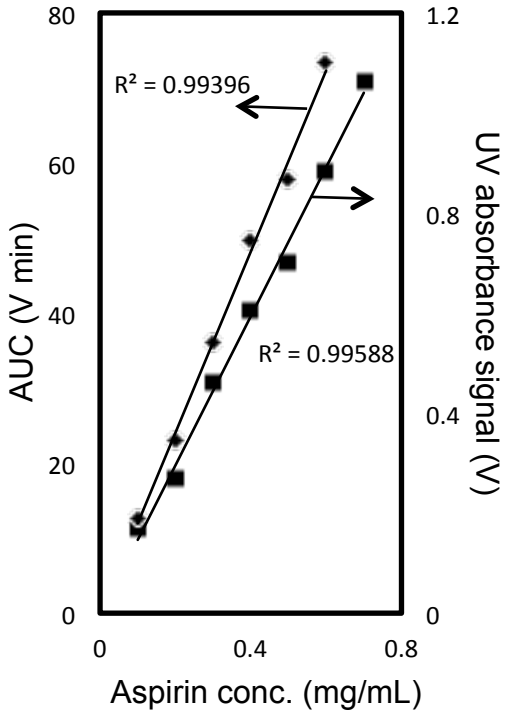

Figure 3.3 Calibration curves in UV absorbance (peak height) and AUC. Both show acceptable linearity.

In figure 3.3 the signals were within the linear range for aspirin in both the AUC and peak height measurements when the concentration was between 0.1 to 0.8 mg/mL in PBS. In Figure 3.4, for the clarity of the figure, experiments of aspirin of 0.1, 0.3 and 0.6 mg/mL were selected to show a significant binding effect to BSA in PBS. As the BSA samples were made into batches with lower (4 mg/mL) and higher (40) concentrations in the hope of revealing the stoichiometry of the binding. It can be found in Figure 3.4 (A) and (B), the amplitude of decrease in permeate peaks of higher BSA binding is much larger than those of their lower concentration counterparts.

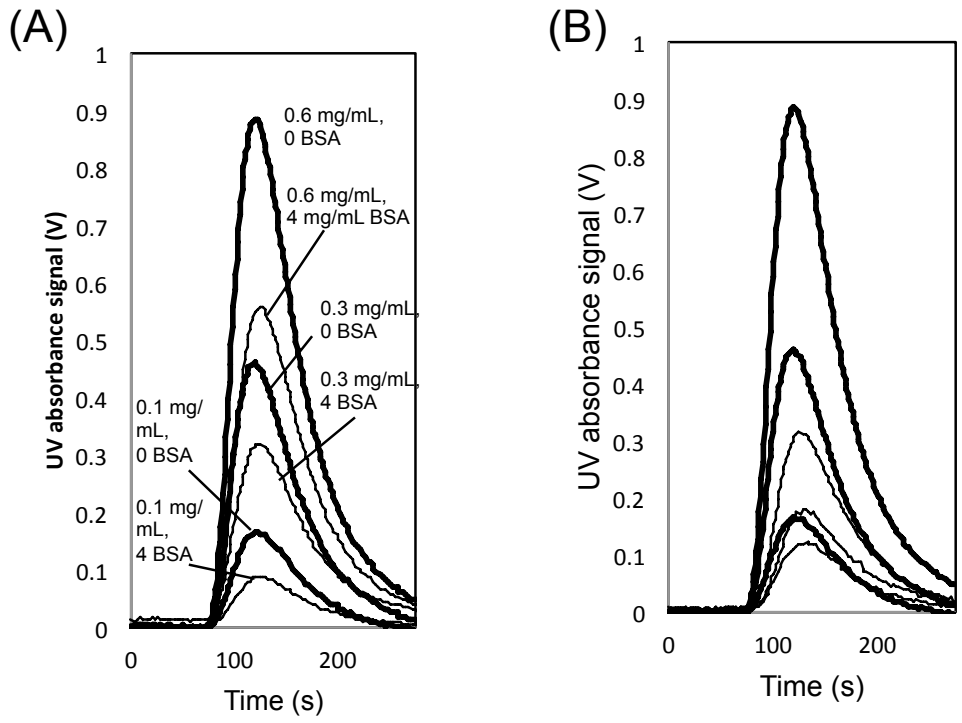

Figure 3.4 Binding of aspirin to 4 mg/mL (A) and 40 mg/mL (B) BSA. 0.1, 0.3 and 0.6 mg/mL peaks were chosen for clarity of view.

In the subsequent Figure 3.5, the fractions of bound aspirin were scattered in to the chart against the total (A) and free (B) aspirin concentrations in both the AUC and peak height interpretations. In Figure 3.6, the same plotting was done to the molar ratio of bound aspirin and total BSA as functions of total (A) and free (B) aspirin. For a clear view, some squares are transformed into crosses and diamonds into ticks where the legends are otherwise crowded.

In Figure 3.7, continuously repeated peaks were reported to visualize the reproducibility of the system under the noted experimental conditions. A more extensive survey was done and results compiled into Table 3.1, including conditions where the system failed.

Assuming the binding of drugs to BSA is in equilibrium, the free drugs can be extracted for detection from the incubate by ultrafiltration as the proteins will be held back due to their size. The tangential flow set up is good in terms of sample elimination, as the retentate will be eventually washed off to the waste tank. It offers facility for a single

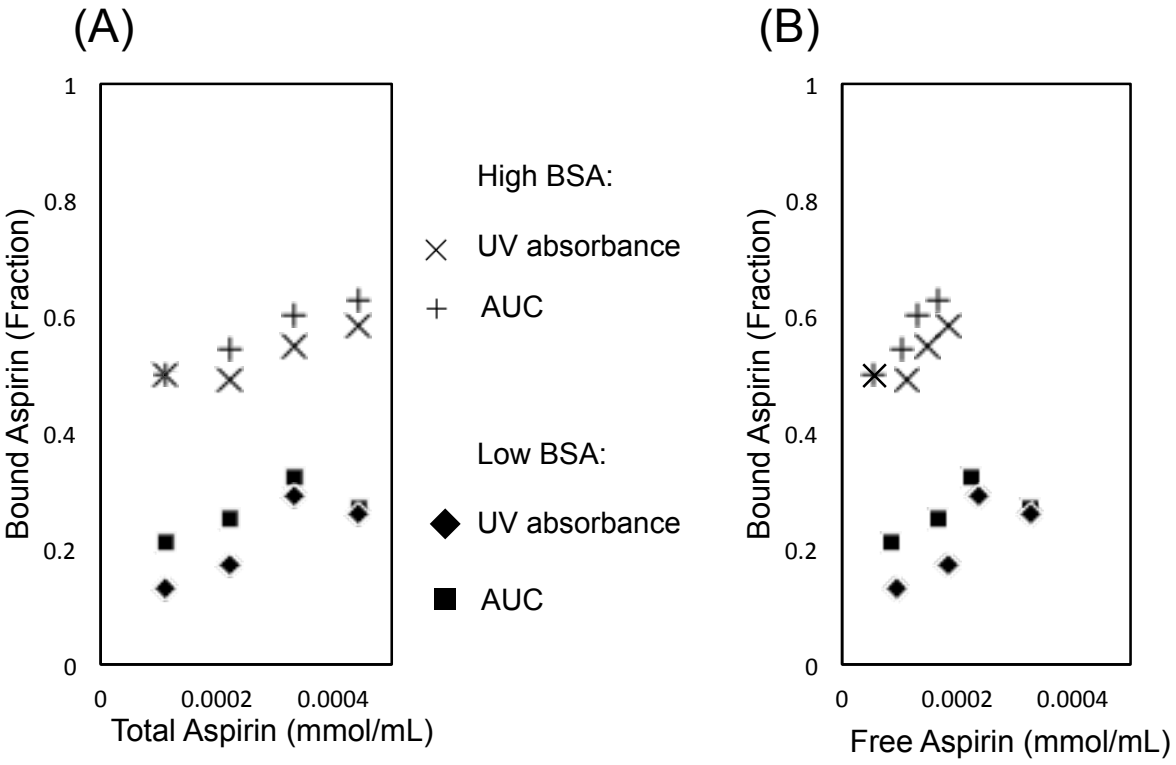

Figure 3.5 Fractions of aspirin bound as functions total (A) and free aspirin (B). Showing an increase of binding with 40 mg/mL (High) BSA over to 4 mg/mL (Low) BSA.

installation to test multiple samples between which a simple wash is carried out without replacing the membrane. The relatively short exposure to the suction suppresses the

equilibrium adsorption of the drug to the membrane. The entire process is done on a scale of minutes. However, the sensitivity and reproducibility are the key factors to assess the system as an alternative analytical method.

In Figure 3.3 the linearity of aspirin samples from 0.1 to 0.8 mg/mL was also observed for both the AUC and peak height measurements. Binding experiments were carried out using two a higher (40 mg/mL) and a lower (4) concentration of BSA. In Figure 3.4 (A), the binding was clearly shown by the decrease in UV signal in 0.1, 0.3, 0.6 mg/mL of aspirin incubated with 4 mg/mL BSA.

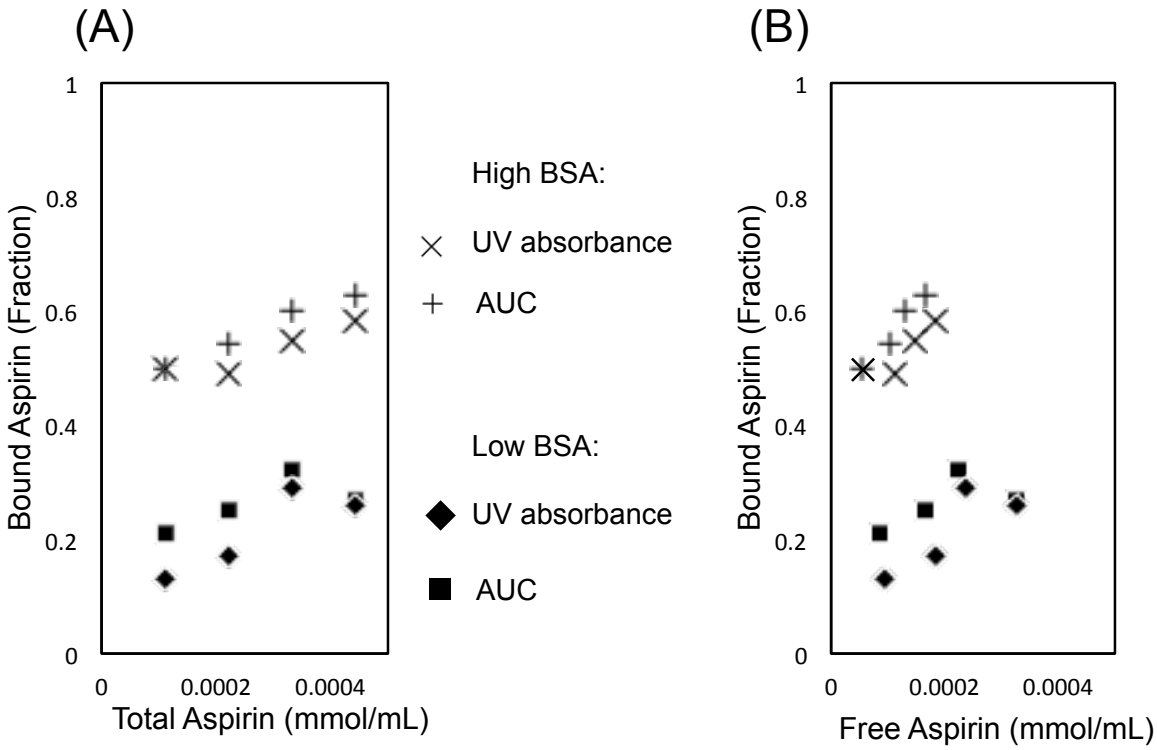

Figure 3.6 Mole of aspirin bound to per mole of BSA as functions of total (A) and free (B) aspirin.

In Figure 3.4 (B), with a higher concentration of BSA the peaks of the free drug decreased significantly, as expected due to the shift of equilibrium towards more binding. In theory, as the concentration of the drug increases, the equilibrium will also shift towards the binding side. Data in Figure 3.5 (A) revealed a clear increasing trend in fractions of bound aspirin as the concentration of aspirin increased. Lower BSA concentration lead to a milder increase. Under such condition, the aspirin saturated the binding sites available from 4 mg/mL BSA more easily whereas the more abundant binding sites presented by the 40 mg/mL BSA will passivate this effect so that the fractions of bound increased in a steeper profile. However, as the mass conservation of the drug dictates, an increased binding fraction, as in the 4 mg/mL BSA experiments, will greatly reduce the fraction of free drug. As a result, the fractions increased in a sharper profile as a function of free aspirin concentration in Figure 3.5 (B). Moreover, the x position of the data points of the higher BSA concentration experiment were clustered to the left side as opposed to that those with total aspirin as x axis in Figure 3.5 (A). The trends in AUC and peak heights agreed in both plottings.

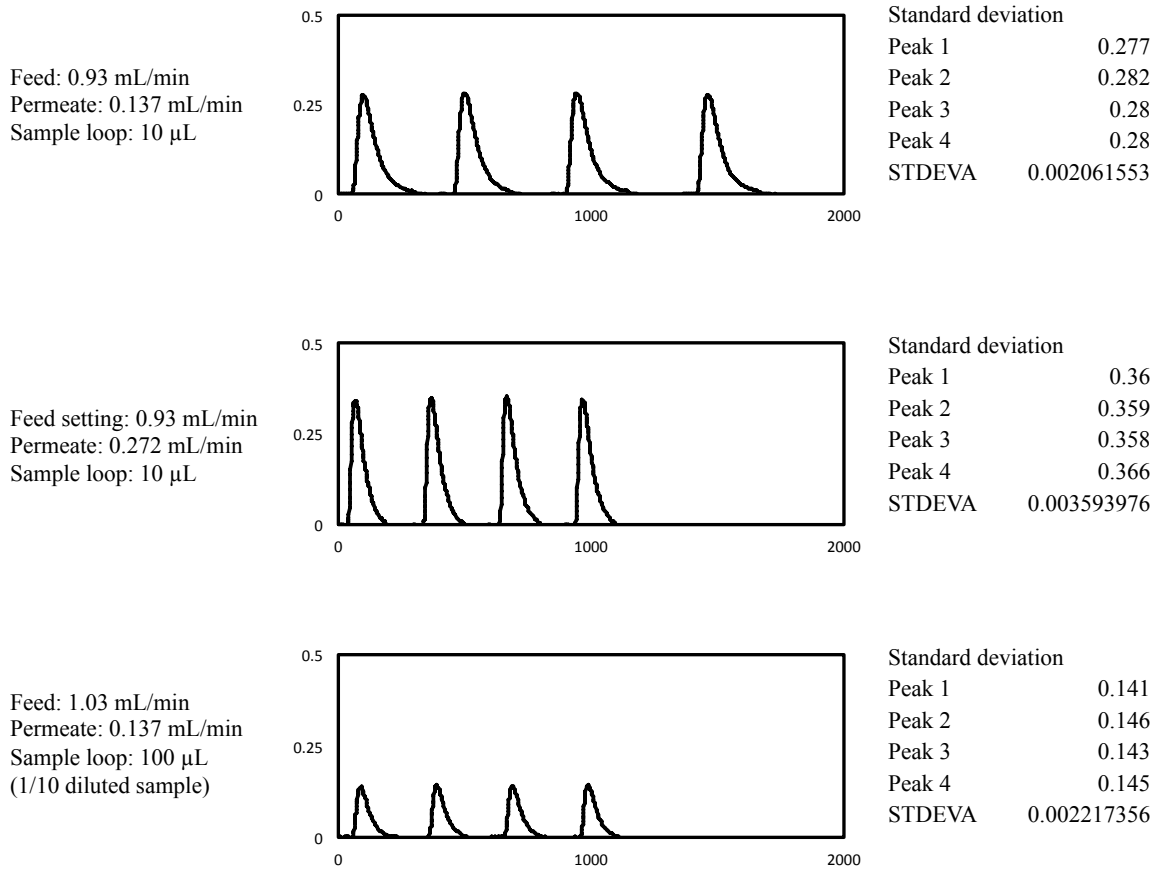

Figure 3.7 Reproducibility of signals under different parameter settings

In Figure 3.6 the stoichiometry of the binding of aspirin to BSA was studied by plotting the molar ratios of bound aspirin to BSA against the total injected (A) and free aspirin in the permeates (B). The trend revealed that the more aspirin added the more it bound to BSA. This effect became more significant in the experiments with the higher BSA concentration. It was suggested in Figure 3.6 (B) despite of different BSA concentrations, the binding ratios correlated to the detected free aspirin concentrations in the incubated samples in a similar slope. The latter may be the more representative way to interpret future data.

Permeate	Feed	Retention time	Peak height	Peak width	Notes
mL/min	mL/min	seconds	voltage	seconds	
0.1356	0.93	*140*	0.355	202	
0.1356	0.465	*106*	0.323	309.7	
0.1356	1.395	*90*	0.206	158	
0.2034	1.395	*81*	0.251	144.7	fastest of all
0.2034	0.93	100	0.27	175	fastest without baseline drifting
0.1017	0.93	162	0.22	301	finest shape
0.2712	1.860	N/A	N/A	N/A	too much disturbance in singal, leaking at inlet
0.4082	1.395	52	0.335	127.7	leaking at inlet, numbers meaningless
0.678	1.395	N/A	N/A	N/A	bubble forming in permeate line
0.4082	1.395	98	0.252	140	Baseline didn't level

Table 3.1 Flow paramters and performances of the set-up

As in Figure 3.7, the reproducibility of the signal produced with this system were assessed with repeats of a same sample at different conditions. A common scale was used to illustrate the time span of the experiments and the significance of the signals. The results were plausible in that the standard deviation is small compared to the amplitude of the peaks in all three conditions. A 1/10 dilution was carried out in a 100 μL sample loop, which is 10 times in volume of the loop used in the rest of experiments. The signal to noise ratio reduced thus greatly providing an insight of using the higher concentration of the chromophore is favorable in the category of detection using such a set-up. It is also in synergy that higher concentration of drug used can reduce the factor of its non-specific binding to the membrane. By doubling the permeate flow rate, the time for the same experiment also reduced to half without compromising the signal strength. On the contrary, the peak height practically increased by a small portion, probably due to the relative flow rate of the permeate side increased with the feed rate kept constant.

Some fine change of the flow rates in both the feed and the permeate line were made to narrow down the most dominant factors for the retention time and peak heights to the permeate flow rate and the concentration of the injected sample among the dead volume of the permeate line, feed rate, loop size, etc. The detailed data were compiled in Table 3.1. As a preliminary set-up, some boundaries of operating parameters were also encountered during the pilot tests. Out of the consideration of a sound chromatogram the baseline drift were unacceptable when the permeate flow rate exceeded 0.4082 mL/min. In addition, due to the nature of suction and the connection, the absence of the flow regulation by the flow-meter down stream on the retentate line will lead to bubble formation in the permeate line. While the feed rate by increasing the pressure, although to a very limited extent, on the feed side will counteract the bubble formation, the exposure time of the analyte to the membrane greatly reduced as an undesirable tradeoff. One challenge remains, as the sensitivity of the UV detector is a limitation to the judicious determination whether a specific drug molecule is suitable for test by a given UV detector.

3.5 Conclusion

Pulsed injection and tangential flow ultrafiltration were combined together as a potential alternative to study drug-protein interaction. Pulsed injections of samples of the same composition gives repeating results with low standard variation. The performance of the prototype system can be fine-tuned by varying the flow rates in the feed line and retentate line, carrying similar variations in precision. Detailed performance data were recorded for reference. Tangential flow granted the system fast and accessible regeneration for

consecutive runs. Aspirin was chosen as the model drug and calibrated giving a linear standard curve. BSA as the binding protein was incubated with the drug. Incubates were extracted of free drugs by the tangential flow module and measured for UV absorbance. Results provided evidence on aspirin binding to BSA in PBS: the binding efficiency of unit BSA is lower in samples where BSA concentrations were higher; aspirin binds more to BSA as its concentration increased while the fraction of aspirin bound decreased. Suitable drugs could be studied by this method, if further developed, for the binding behaviours and as the analytical tool proposed in this chapter is low-cost, a wider distribution could ideally be anticipated.

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4. Analytical applications of membranes as a barrier

4.1 Abstract

Membrane used as selective barriers are often accompanied with naturally occurring accumulation related phenomena. When the solute is entrapped in the pores or surface of the membrane, fouling takes places. Fouling reduces the permeability, increases energy consumption, brings risk of high pressure and may disable the membrane in serious conditions. Nevertheless, the detail of the fouling process may provide useful information in other cases. Concentration polarization, often happening with ultrafiltration of macromolecule solutes, serves as similar drawbacks as fouling on the performance of the filtration. Yet, being a redistribution of solutes, it is reflective of factors such as the size of the molecule and the mode of the flow. Changes in these aspects would likely to place an impact on the profile of concentration polarization. If the accumulated molecules can be detected, it is useful to examine different concentration polarizations with controlled variables to determine the changes in properties in the flow or of the solute. Fingerprints were obtained using various membranes as a demonstration of biometric collection of fine details. Latex beads coloured with deep blue were used as the contrast agent. The different concentration polarization under similar conditions were also visualized using the same contrast agent.

4.2 Introduction

Fouling and concentration polarization are well known under research for their effects in ultrafiltations [1]. Fouling happens when the feed contains particles depositable to membrane surface or within the pores. Several mechanism has been proposed in order to better understand and prevent fouling in most of the established applications[2-3]. Upon fouling, the pores are covered by accumulated inpermeable particles thus unaccessible for subsequent input to the membrane. Only the unblocked pores serve as the original purpose of the membrane as a filter. In cases of large-scale reduction of open pores, the overall permeability will decrease. In process where the flow rate is kept constant, this could result in an increase of pressure and bring about safety issues. Nonetheless, due to the presence of selective passage, membranes fouled in a controlled manner could be useful when revealing the patterns of fouling is of interest. Concentration polarization shares similarities with membrane fouling in terms of solute distribution. Within the polarization layer, the concentration increases substantially towards the membrane surface. Osmosis based phenomena will be present in such cases, resulting in increased processing pressure, decreased overall efficiency and if the feed is dilute, the decrease in the concentration in bulk solution [3-6].

The nature of these phenomena is the dynamic distribution of solutes during ultrafiltation and microfiltration. Some unconventional use could be applied using fouling and concentration polarization. For fouling, if the foulant is stable and can be detected or contrasted patterning involving membrane fouling could be feasible. One advantage is for microfiltration and ultrafiltration membranes the pore size is in the scale of nanometers

and micrometers resulting potential high resolutions [7]. This technique can be used to generate fine patterns on membranes. Generally, a blocking agent is applied to draw the preliminary pattern followed by another round of deposition that is incompatible with the previous agent to reveal its presence. In other case, the blocking agent serves as a precursor for its easy transferrable property or to compensate the deposited material in rheology if the latter is unable to be transferred alone. Then the blocking agent is degenerated as needed. A simplified visualization is shown in Figure 4.1. One example of such technique is the capture of fingerprints. Fingerprints are key to biometric identification processes [8]. In most of the cases, they are obtained by ink printing, optical devices or piezoelectric devices, where limitations apply. Ink printing is subject to smudging and the participant may find the process unappealing by dipping fingers with ink. Optical devices are sensitive but fragile at the same time. Moreover there is outstanding issue of counterfeit fingerprints. While piezoelectric devices are more secure and accurate, the lifetime of such devices is relatively shorter. On the contrary, membranes are clean of germs, robust if handled properly, and in a disposable fashion with little prerequisite investments [9-12].

For concentration polarization, while mostly it hinders the filtration process at least on the efficiency, the behaviour of accumulation of solutes can sometimes be informant about the fine details of the process. Detectable solutes can act as probes to make the regime of the flow apparent for visualization, thus be helpful in revealing hydrodynamic details of the fluid. As the polarization is often a result of some properties of the solute, if the polarization can be quantified, useful information such as size of the solute can be

obtained. For example, in the antibody-antigen interaction, the size of the complex can be well different from the nature antibody. The antibody, if tagged or visualizable, could lead to different pattern of polarization. If incorporated with microfabrication, small form factor devices for assessing affinity between entities of marcomolecules are possible.

In this chapter, fingerprints are obtained by entrapping body grease naturally found on fingertips to microfiltration membranes. The simplicity of equipment, resolution, overall cost efficiency and limitations are demonstrated. Dyed particles are employed in revealing subtle differences during ultrafiltrations from the same module with different level of tightenedness of the assembly. Visual evidence was collected suggesting for extra attention when the results are prone to change with the fluid mechanism of an experiment. Even with seemingly identical operations, the nature of the flow could be different.

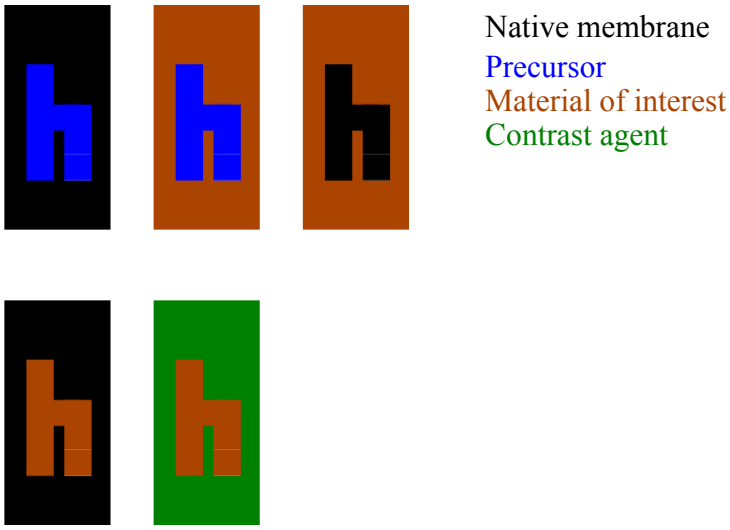

Figure 4.1 Working principle of pattern formation on membranes. Negative (top row) and positive (bottom row) pattern transferring techniques.

4.3 Experimental

4.3.1 Materials

All membranes were purchased from Pall Inc. and used without further treatment with the consideration of easy accessibility by employing commercialized products. Latex beads – deep blue dyed of 0.8 micrometer and ferritin from horse blood were obtained commercially from Sigma-Aldrich and diluted with deionized water before use. P-50 displacement pump was purchased from Amersham (GE Healthcare Life Sciences). Ismatec Quick-Couple Programmable Digital Drive was purchased from Masterflex (Cole-Parmer). Modules were custom-designed and fabricated.

4.3.2 Fouling experiments

Clean finger involved in daily academic activity was used for transferring fingerprint to microfiltration membranes of different materials. Generally, the finger was pressed against the membrane for a reasonable period of time around ten seconds comparable to that done in a typical fingerprinting collection by other common means. Then the same membrane was installed in the membrane module to seal properly such that all feed was forced through the membrane and the latex particles deposited on the membrane as fouling. After the water has drained the module was disassembled and the membrane with contrasted fingerprint obtained. Pictures of these membranes were taken as a way of digitization of the information. For ethic concerns only partial area of a single print in each experiment was used in order to show the resolution and pattern capture of the technique.

4.3.3 Concentration polarization using TFF module

The module is constructed by a top part and bottom part combined together by eight screws, with one gasket in between to make sure a channel is secured when the module is tightened up. A 10k ultrafiltration membrane was fit under the slit of the gasket and be pressed by the gasket and the bottom part. The inlet and retentate were connected to the Masterflex pump with a reservoir for the circulation of solution. The permeate was drawn by the P-50 pump for a more precise suction. The module was filled with water before the latex beads were introduced. The latex bead sample was diluted to one twentieth of its original concentration. Pictures of patterns were taken by Panasonic DMC-FP3 camera.

4.3.4 Concentration polarization using stirred cell

A stirred cell with two lenses mounted for laser beam was custom fabricated to maximize the membrane area and minimize the volume of the cell to the extent allowed by the fabrication specification. A laser was shined through the bulk of the solution to monitor the change in absorbance of the ferritin. When a flux is on, the bulk signal decrease was analyzed to reveal the extent of concentration polarization. Ferritin absorption at 280 nm was extracted from spectrum data as the flux increased. Discrete points were obtained instead of a profile revealing real time concentration polarization, due to the fact the software setup was still under development.

4.4 Results and discussion

In Figure 4.2 the fingerprint on a PES membrane was magnified as a demonstration of the bifurcation. Although the lipids from finger tips are not usually excessive that it is so obvious in daily activities, the fact that microfiltration membranes have very fine pores makes them very sensitive to oil foulants. A firm and brief touch was able to transfer the pattern of fine structure of fingerprints to the membrane surface by selectively blocking the pores where the ridges of skin are present. The affinity of body grease is high over organic membranes so the absorbed lipid material doesn't spread easily. As the membrane is hydrophilized, the water will go through the membrane from the unblocked pores leaving the fingerprint with little flux. The latex in the water feed is thus deposited where there is significant water flux, giving a contrast to the lipid-rendered area. A fingerprint of blank colour is then available for photographic recording into databases. The entire process is non-invasive to the fingerprint supplier with no artificial material added to the surface of the skin. The availability of the body lipid is likely to be more than sufficient in most of the cases. In case there is not enough grease to transfer a clear print, the supplier could take up lipid from forehead or cheek area. However, the binding of the latex to the surface of membrane varies with the combination of the material of the beads and membrane.

A draw back of PES membrane is the pattern is easily rubbed off after the filtration has been done. This is a set back when the information is collected by scanning. An

alternative material, cellulose acetate was found to provide resident deposition of latex beads without any noticeable change in resolution and fineness of the patterns obtained as shown in Figure 4.3. In contrast with piezoelectric devices, this proposal uses a less costly set of equipment while still providing acceptable accuracy for archiving biometric information. This gives it potential of being widely deployed to area where the cost of the instrumentation should be minimized. On the occasion where the germ-related concerns, this solution could also be an alternative where inks are still used to collect fingerprints as an offer to relieve the concern of the participant.

Figure 4.2 A contrasted image of a partial fingerprint captured on PES membrane. Details such as bifurcation are seen clearly. Positive pattern transfer.

In Figure 4.4, the concentration polarization was shown as blue deposition on the surface of the membrane near the center of the module where the opening on the support is present. When the membrane is tightened to an extent where the leakage was just

eliminated from the size of the module, the pattern is repeatable and identical between

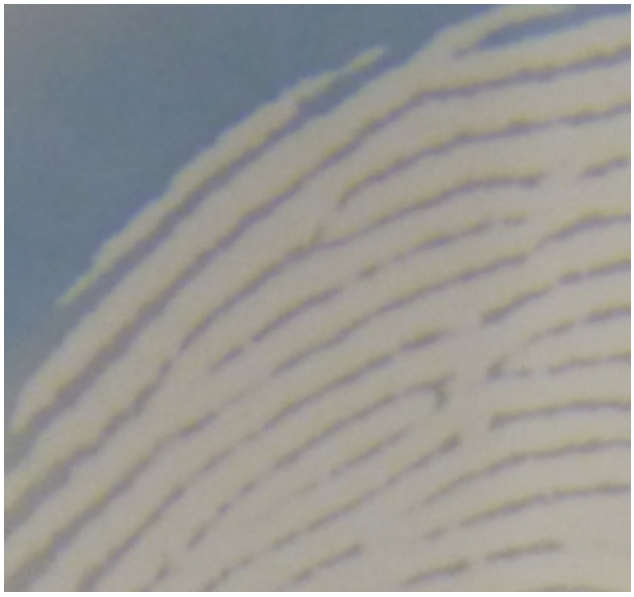

Figure 4.3 Same fingerprint captured on CA microfiltration membrane. As the imprint was resistant to scrubbing, light smudging between lines was observed. Positive pattern transfer.

runs. In order for the explanation of this phenomenon, it is hypothesized that if there is an allowance of space for the membrane to adjust, it will stay in a configuration that the net flow is maximized under the same amount of driving force. Instead of sitting flush against the bottom compartment, the membrane bulged up to allow a bigger area than the opening on the support so that more of the feed could go through. As the geometry of the opening suggests, the flux is present in a radially equal distance from the center, the vector of flow at the center of circle is cancelled by the joining flux, creating a stagnant area. The reduction of flux in this scenario is not relevant in terms of performance of filtration but rather a way to reveal the specific condition of flow under such condition.

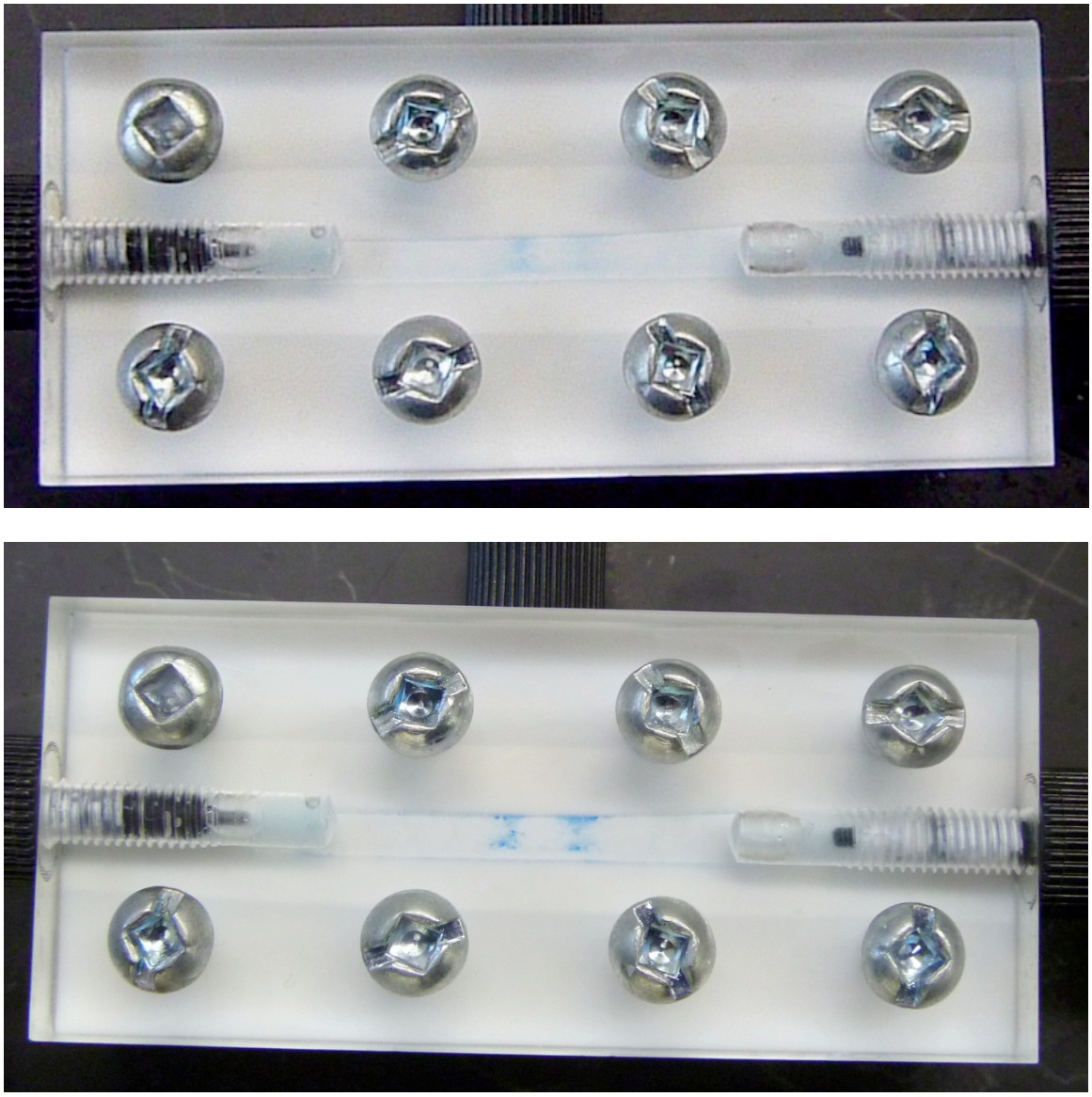

Figure 4.4 Development of concentration polarization with stagnant area. The module tightened to provide free space allowing the membrane to adjust.

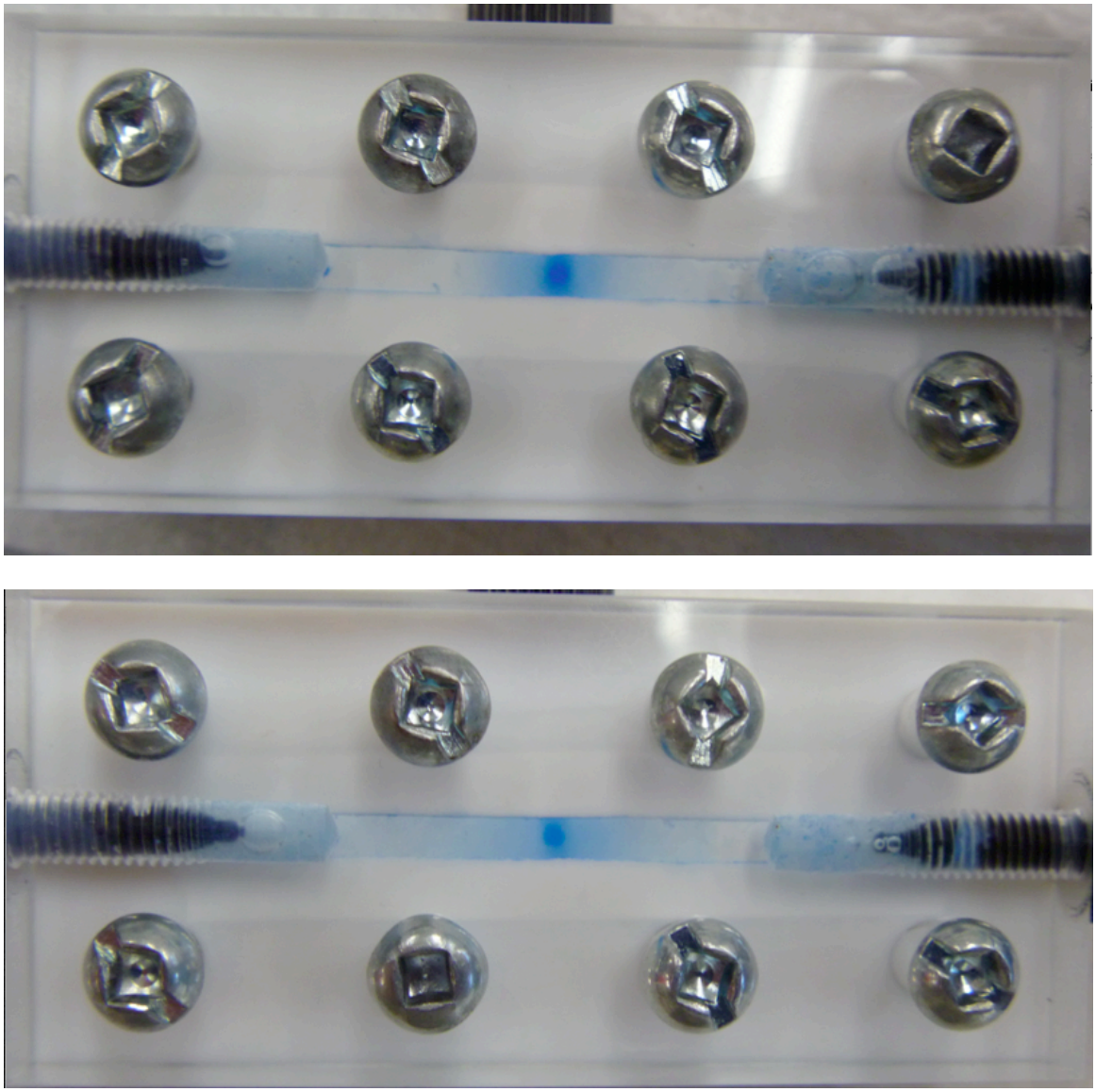

Figure 4.5 Development of concentration polarization with no stagnant area. The membrane was confined between the top and the support.

To further substantiate the hypothesis, the same module and membrane was set up with the screws tightened to a much larger extent such that the membrane is sealed against the support and little liquid should go through. The deposition is shown in Figure 4.5 demonstrating a circle of blue deposition identical to the opening with a faint coloration

around it. This suggests in the condition of restricted flow, the most concentration polarization will still occur intuitively over the space where there is opening and accessible membrane area.

4.5 Conclusion

Fouling and concentration polarization in membrane filtrations were examined from a perspective that the utility of such phenomena is essentially relevant. Invisible foulant such as body lipid can be contrasted on a membrane surface to reveal fine details as subtle as fingerprints. A means of biometric informatics application was suggested. Colorations of the concentration polarization of dye particles was used as different flow modes in the same module with slight difference on how the module was tightened. The properties of solute and the membrane as well as the nature of flow should receive further exploration since the preliminary results from this chapter strongly hint the existence of strict logic and mechanisms behind.

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5. Conclusions and recommendations

5.1 Conclusions

At an overview of this thesis, the versatility of membrane science and technology was applied to various researches including bioseparations, biomedical and analytical applications. The following conclusions are drawn based on the experimental results in chapters two, three and four.

- The challenges of unexpected solute transmission in the microfiltration of PNIPAM microgels were addressed and a successful purification was demonstrated.
- The salt-responsive transmissions of microgels were studied under fixed and sweeping parameter conditions in microfiltrations.
- The transmissions of microgels in microfiltration and ultrafiltration were studied.
- A hypothesis of microgel being extensively deformable was raised and verified by increases in transmission in changing fluxes microfiltrations.
- A prototype analytical method utilizing pulse tangential ultrafiltration was developed. The binding of aspirin to BSA was evaluated using this method.
- One of the appealing performance factor of the proposed set-up is its ability to process samples at high rate.
- Fingerprints were captured on microfiltration membranes fouled by body lipid present on the skin of fingertips and contrasted by colored deposition

incompatible with such lipids.

- Concentration polarization of dye latex beads against ultrafiltration membrane was employed to reveal the possible self-adaptation of the membrane in order to maximized the area of flux and minimize the resistance in ultrafiltration.

5.2 Recommendations

On top of the conclusions in previous section, there are several recommendations for prospective research and applications.

Regarding the topic of chapter two, possible fast purifications of microgel and other microscopic biomedical composite products is feasible for lab environments. Standardized equipment could be widely distributed as an alternative where dialysis is traditionally used. The transmission profiles can be useful in investigations where structural changes of soft nanoparticles without changing the apparent hydrodynamic size. As for most current measurements, the detailed information of the structure of such particles are still challenging to provide. The salt responsive behaviours could be expanded into responsiveness to pH and other environmental parameters to provide analogous prediction to the conditions in vivo where pH or ionic strength varies between tissues and organs.

The analytical method described in chapter three could see great potential of being further developed into one with mature performance. The quick and easy regeneration of the instrument to a ready state allows saving of operation attention and possible automation of multiple samples. The fast analysis could also be used to gather kinetic information of

drug binding to proteins. With a similar configuration, protein molecules could be adsorbed to certain sorbents that are retained by the membrane while the free protein detected to analyze the protein-sorbent binding behaviour. To a more generic scope, any detectable analyte with binding to a substrate could be studied using TFF set-ups for fast accessible inquisitions. For example, the dynamic impurity level in the waste of a mixture being purified could be obtained automatically for a semi-real time result in an unattended fashion.

The fourth chapter provided an arguable new direction of membrane fouling and concentration polarization. More patterns could be transferred to membranes with microscopic pores due to their high tendency of being blocked. For example noise prints of household pet dogs could be obtained in a less invasive way without putting any extra material to the nose the animal, thus reducing the potential unexpressed discomfort of the process. Fluorescent particles could also be used in revealing the local concentration polarization profile given the sensitivity of fluorescence is substantially higher than the reflective lights. The applications could further reduce into micro and nanoscale, finding similar applications in microfluidics, where as the channel are more confined, the local flow mode bears more chance to influence the final results. While in concentration polarization the accumulation of solutes on the surface of membrane could in some cases result in a significant decrease of bulk concentration if the solute can be detected. This could be potentially convenient if the solute forms into complex and changes the effective size when a specific binding agent is introduced.