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An osmolyte-based micro-volume ultrafiltration technique

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Abstract This paper discusses a novel, simple, and inexpensive micro-volume ultrafiltration technique for protein concentration, desalting, buffer exchange, and size-based protein purification. The technique is suitable for processing protein samples in a high-throughput mode. It utilizes a combination of capillary action, and osmosis for drawing water and other permeable species from a micro-volume sample droplet applied on the surface of an ultrafiltration membrane. A macromolecule coated on the permeate side of the membrane functions as the osmolyte. The action of the osmolyte could, if required, be augmented by adding a supersorbent polymer layer over the osmolyte. The mildly hydrophobic surface of the polymeric ultrafiltration membrane used in this study minimized sample droplet spreading, thus making it easy to recover the retained material after separation, without sample interference and cross-contamination. High protein recoveries were observed in the micro-volume ultrafiltration experiments described in the paper.

Introduction

Rapid developments in the field of proteomics have been fuelled by improvements in analytical techniques[1] and widespread application.[2] Also, innovations in areas such as separation science and technology, and microfluidics have made it possible to develop new techniques, and fabricate efficient devices for proteomic analysis and related applications.[3-8] Protein purification processes such as concentration enrichment, desalting, and buffer exchange, typically carried out for sample pre-treatment during proteomic analysis, should ideally be able to purify a large number of micro-volume samples in parallel, in an automated fashion. Micro-volume protein separation is most commonly carried out in the high-throughput mode using separation techniques that are compatible with 96 and 384 microwell plates.[9-12] It is conceivable that in the future, such separations may need to be carried out in formats suitable for processing even larger number of micro-volume protein samples.

The most popular methods currently used for high throughput protein separation are centrifugal ultrafiltration[13-16] where the transmembrane pressure needed for driving flow of liquid through the membrane is generated by centrifugation, and vacuum ultrafiltration[17, 18] where suction is applied to the permeate side of the membrane. Other techniques used for micro-volume protein separation include micro-dialysis[19, 20] and micro-column based separations which can process samples down to a few microlitres.[21-24] Some of these currently used techniques require sophisticated and expensive equipment such as specialized centrifuges. Others involve protocols which are either slow, or not particularly amenable to automation, or use expensive columns and reagents. Column based desalting techniques are also time-consuming, and usually result in sample dilution. Currently used micro-volume protein separation techniques are generally difficult to monitor as the samples being processed are neither visible nor accessible during processing. Moreover, most of these techniques do not work efficiently with very low sample volumes, i.e. less than 10 microlitres, as most of them are designed and developed for handling samples in the several 10s to several 100s of microlitres range.

This paper describes a novel, simple and inexpensive micro-volume ultrafiltration technique, particularly suitable for carrying out protein separation in the less than 10 microlitre range. It could be used for protein concentration enrichment, desalting, buffer exchange, and for size-based protein purification. The technique is amenable to automation and could be carried out in a high-throughput processing mode. Moreover, the protein samples are visible and accessible during purification and process monitoring is therefore feasible. The transmembrane pressure for ultrafiltration is generated by a combination of capillary action, and osmosis. As shown in Figure 1, water and other permeable species are drawn from a micro-volume sample droplet applied on the surface of an ultrafiltration membrane utilizing a macromolecular osmolyte such as poly (ethylene oxide) (or PEO) coated on the permeate side. If required, the action of the osmolyte could be augmented by using a supersorbent polymer such as the sodium salt of poly (acrylic acid) (or PAA), and the efficiency of the technique could thereby be further enhanced.

The micro-volume ultrafiltration membranes used in the current study were prepared by coating either PEO alone, or a combination of PEO and PAA on the permeate side of polyethersulfone (or PES) ultrafiltration membrane having molecular weight cut-off (MWCO) of 10 kDa. PEO served as the primary water-drawing osmolyte while PAA served as a supersorbent polymer for increasing water uptake on the permeate side. The PES ultrafiltration membrane used in this

study could be used by itself to remove water from an applied protein sample droplet, purely by capillary action. However, the presence of the osmolyte, or the osmolyte-supersorbent combination significantly increased the rate of water removal, and hence the speed and efficiency of separation. The different membranes described above were used to concentrate and desalt different micro-volume protein samples. The results thus obtained are discussed.

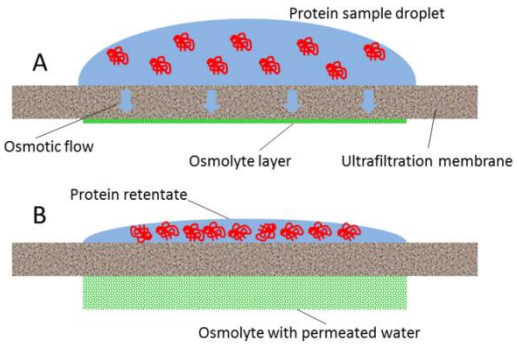

Fig 1 Working principle of osmolyte-based micro-volume ultrafiltration (A: sample droplet applied on the skin-side of the membrane; B: concentrated protein solution retained on the membrane due to water removal by osmolyte).

Experimental

Materials

Bovine serum albumin (A-2153), lysozyme (L-6876), FITC albumin (bovine albumin fluorescein iso-thiocyanate, A-9771), poly (ethylene oxide) (PEO, average Mv ca. 200,000, 181994-250G), poly (acrylic acid) partial sodium salt (PAA, <1000 μm particle size, 436364-250G) and other chemical were purchased from Sigma-Aldrich, St. Louis, MO, USA. Monoclonal antibody hIgG1-CD4 (Batch 12) was kindly donated by the Therapeutic Antibody Centre, Oxford, United Kingdom. Purified water (18.2 MΩ cm) used in this study was obtained from a DiamondTM NANOpure (Barnstead, Dubuque, IA, USA) water purification unit. Polyethersulfone (PES) ultrafiltration membrane sheets (10 kDa MWCO, OMEGA, part OT010SHEET, lot H1173E) were purchase from Pall Canada, Mississauga, ON, Canada.

Preparation of PEO coated membrane

Osmolyte coated ultrafiltration membranes were prepared by coating PEO on the permeate (or rough) side of a rectangular (ca. 4 cm × 6 cm) piece of 10 kDa MWCO PES ultrafiltration membrane. The PEO solution (100 mg/mL in purified water) was applied as a thin layer on the membrane using a paint brush. The coating solution which was viscous formed a uniform layer on application but had a mottled appearance during drying. After drying, the osmolyte layer looked more or less uniform. The membrane piece was dried at room temperature for a minimum of 48 hours before being used in ultrafiltration experiments.

Preparation of PEO and PAA coated membrane

PAA sodium salt used as the supersorbent polymer was ground into a fine powder using a ceramic mortar and pestle. PEO solution (100 mg/mL in purified water) was coated as a thin uniform layer on the permeate side of a piece of membrane (ca. 4 cm × 6 cm). About 30 seconds after applying the PEO coating, the PAA powder was sprinkled on the still wet membrane surface in the form of a thin layer of ca. 0.25 mm thickness. The membrane piece was then dried at room temperature for a minimum of 48 hours before being used.

Micro-volume ultrafiltration experiments

96 holes having ca. 7.5 mm diameter were drilled in a 127 mm × 85 mm ABS plate (Easy-to-Machine Impact-Resistant, black opaque, 1/8" thick, part number 8586k161, McMaster Carr, Cleveland, OH, USA). The holes were arranged in 8 rows of 12 in each, with their centres 9 mm apart, corresponding to the format of wells in a standard 96 micro-well plate. Membrane discs of ca. 8.5 mm were cut out from the membrane sheets using a steel hole cutting punch. These discs were attached to the ABS plate by gluing them around the rim of the holes using Glue-All polyurethane glue (Elmer's Canada, Markham, ON, Canada). Micro-volume ultrafiltration experiments using the 10 kDa PES membrane as well as those using the PEO coated PES membrane were carried out using such 96 membrane disc assemblies (see Figure 2). The experiments using the PEO and PAA supersorbent combination coated membrane were carried out with square pieces of membrane of ca. 10 mm × 10 mm dimension as these membranes were difficult to cut into discs using the steel punch.

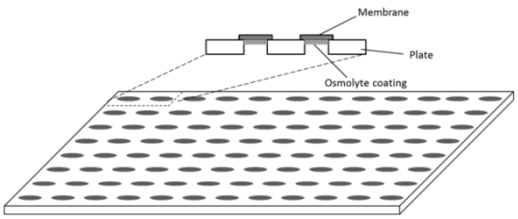

Fig 2 96-well membrane format used in micro-volume ultrafiltration experiments.

The protein samples for micro-volume ultrafiltration were applied as droplets using a pipette on the skin (or shiny) side of the membrane. Water and permeable species were drawn through the membrane, leaving behind a layer of concentrated protein solutions on the membrane surface. The surface of the PES ultrafiltration membranes used in this study being mildly hydrophobic, the sample droplets did not spread to any significant extent on the membrane surface. This surface property also made it easy to recover the concentrated retentate by pipetting. In protein desalting, buffer exchange and size-based purification experiments, the liquid lost through the membrane from the sample droplet was replenished by pipetting appropriate volume of buffer over the retentate layer. After performing the required number of such ultrafiltration and buffer top-ups, the final solution was gently mixed by repeated aspiration and dispensing, and then recovered by pipetting from the membrane surface. Photographs and video clips were obtained during the different micro-volume ultrafiltration experiments using a WB700 digital camera (Samsung, Seoul, South Korea).

Protein sample analysis

Protein concentration was determined by UV absorbance measurement at 280 nm wavelength using a NanoDrop 2000 UV visible spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The recovered protein samples were also scanned over the UV visible range to check for aggregation or change in optical properties. The amount of retentate recovered after micro-volume ultrafiltration was measured by weighing the sample using a BL120S analytical balance (Sartorius AG, Gottingen, Germany). The salt concentration in samples obtained from the protein desalting experiments was

determined by refractive index measurement using a hand held refractometer (A300CL-E01, Atago, Tokyo, Japan). Protein samples obtained from size-based protein purification experiments as well as those carried out to check for sample interference or cross-contamination were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).[25] Non-reducing SDS-PAGE (10% for the verifying interference, and 12.5% for checking purity) experiments were carried out using a Hoefer MiniVE vertical electrophoresis unit (GE healthcare Bio-Sciences, Montreal, Quebec, Canada). The electrophoresis experiments were run with normal polarity at 120-140 V (25-50 mA current). Protein bands on the gel were visualized by staining with Coomassie blue dye.

Results and discussion

Table 1 shows the time required for micro-volume ultrafiltration of pure water using the 10 kDa PES and the PEO coated 10 kDa PES membranes. For these water ultrafiltration experiments, the time required was defined as that needed for an applied water droplet to completely disappear into the membrane. With the PES membrane, the water was sucked into the pores by capillary action alone. Evaporation loss was a minor factor, as verified by determining the time required for the disappearance of a water droplet applied on the surface of a non-porous polymer (polystyrene) which gave a comparable droplet diameter and contact angle. At the same conditions (i.e. room temperature, relative humidity and location within the laboratory) as used in the micro-volume ultrafiltration experiment, a 5 µL water droplet took ca. 65-70 minutes to completely evaporate. With the PEO coated membrane, 5 µL of water was completely ultrafiltered in 3.4 minutes which was about 2.85 times faster than that with the uncoated PES membrane, clearly demonstrating the role of the osmolyte in speeding up the ultrafiltration process.

Table 1 Comparison of micro-volume ultrafiltration of pure water using uncoated and PEO coated membranes (data obtained from experiments carried out in triplicate)

Membrane	Time (min)		
	5 µL	10 µL	20 µL
10 kDa MWCO PES	9.7±0.5	18.6±0.6	36.5±2.5
10 kDa MWCO PES coated on the permeate side with 200 kDa PEO	3.4±0.3	6.9±0.8	16.1±1.0

The PES ultrafiltration membrane used in this study was asymmetric, i.e. consisted of a thin and tight nano-porous skin layer on the feed side atop a much thicker and relatively open microporous layer on the permeate side.[26] Most pores in an asymmetric ultrafiltration membrane are aligned normal to the membrane surface. This type of membrane morphology facilitated the development of osmotic pressure between the sample droplet and the macromolecular osmolyte. With membranes having randomly oriented pores, with high degree of interconnectivity, as in most symmetric membranes, any osmotic pressure difference between sample and osmolyte would be difficult to harness. Also, the relatively larger pore diameter on the permeate side of the PES membrane allowed the PEO molecules to enter and coat the walls of the pores closer to the permeate side, during membrane preparation. As shown in Figure 3, water from the droplet applied on the feed side was initially drawn into the pore entrance on the skin side, by capillary action. The presence of the PEO on the surface of the pore wall facilitated its wetting by the influent water, thereby speeding up the penetration of water into the membrane. The influent water then dissolved the PEO molecules creating a highly concentrated osmolyte solution within the pores, and eventually on the permeate side of the membrane. The resultant osmotic pressure difference between osmolyte solution and the feed solution was the primary driving force for micro-volume ultrafiltration with the PEO coated membrane. The osmolyte used in the current study, i.e. PEO was large and did not diffuse into the samples being processed. Significantly stronger osmotic draw could potentially be generated using small molecules as their resultant molar concentrations are higher for the same mass of osmolyte coated. However, these molecules would contaminate the protein samples being processed. In applications where such contaminations pose no problem, small molecules such as glucose and sucrose could be used as osmolyte for micro-volume ultrafiltration.

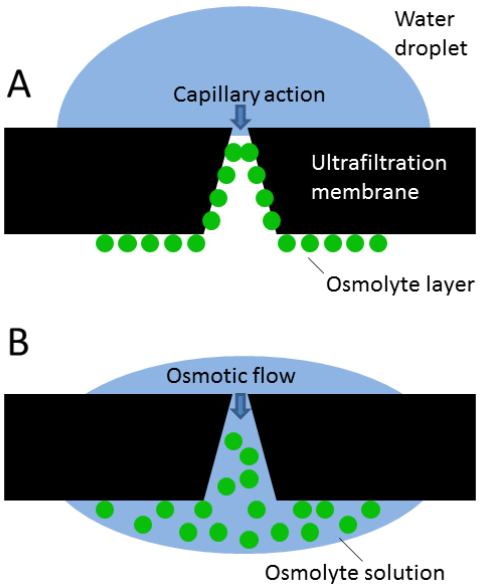

Fig 3 Combination of capillary action and osmotic draw of liquid utilized in micro-volume ultrafiltration.

Figure 4 shows micro-volume ultrafiltration of FITC-albumin solution using the PEO coated PES membrane. Figure 4A shows a droplet of FITC-albumin being applied on the skin side of the membrane; Figure 4B shows the semi-dry residue formed by protein retained on the membrane surface, which eventually formed a dried protein deposit; Figure 4C shows the reverse (i.e. the permeate) side of the membrane after ultrafiltration. The PEO solution formed by dissolution in water removed from the protein sample droplet is clearly visible in the figure. It can also be seen that the PEO solution is colourless, which indicated that FITC-albumin was totally retained on the feed side of the membrane. Table 2 summarizes the time taken for ultrafiltration of FITC-albumin solution using PES, and PEO coated PES membranes respectively. In these experiments, the ultrafiltration time was defined as that required for forming a semi-dry protein residue on the membrane surface. Unlike in the pure water ultrafiltration experiments where the disappearance of a water droplet was a clearly defined event, determining the precise moment when the protein residue was formed was difficult. However, the difference in ultrafiltration time between the experiments carried out using the PES and the PEO coated PES membranes was obvious and significant in magnitude. A comparison of the results shown in Tables 1 and 2 shows that with the PES membrane, the time required for

ultrafiltering the same volume water or protein solution were almost the same. This was because the draw of liquid into the membrane by capillary action was unaffected by the presence of protein in the sample. On the other hand, with the PEO coated PES membrane, there was a significant increase in ultrafiltration time due to the presence of protein. This was because the retained protein molecules near the pore entrance reduced the osmotic draw of liquid into the membrane, this being analogous to the osmotic back pressure observed in conventional pressure driven ultrafiltration. Despite this, the ultrafiltration time observed with the PEO coated PES membrane was much lower than that with the uncoated PES membrane.

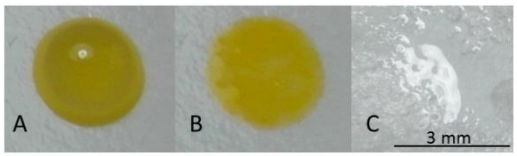

Fig 4 Micro-volume ultrafiltration of fluorescent-labelled protein. A: FITC-albumin solution sample droplet applied to membrane surface; B: retained protein concentrate; C: water/buffer drawn to the other side of the membrane after micro-volume ultrafiltration.

Table 2 Comparison of BSA-FITC micro-volume ultrafiltration using uncoated and PEO coated membranes (BSA-FITC concentration in feed: 2 mg/mL; feed buffer: 50 mM sodium phosphate, pH 7.0; sample droplet volume: 5, 10 and 20 µL) (data obtained from experiments carried out in triplicate).

Membrane	Time (min)		
	5 µL	10 µL	20 µL
10 kDa MWCO PES	9.0±0.3	19.3±0.5	27.6±0.9
10 kDa MWCO PES coated on the permeate side with 200 kDa PEO	5.1±0.4	11±0.3	18.9±0.3

The sequence of events in a typical protein concentration experiment is shown in Figure 5. Figure 5A shows a droplet of FITC-albumin being applied on the surface of PEO coated membrane; Figure 5B shows the droplet immediately after application; Figure 5C show the retentate after concentration; Figure 5D shows the FITC-albumin concentrate being recovered from the surface of the membrane by pipetting. Table 3 summarizes the results obtained from experiments carried out to concentrate 20 µL of 1 mg/mL BSA solution using PEO coated PES membrane. The concentration factor and protein recovery after 6 minutes were 2.38 and 95% respectively, while after 12 minutes, the corresponding values were 9.05 and 90.5% respectively.

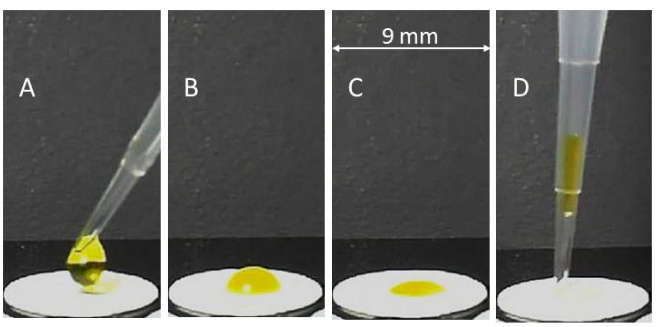

Fig 5 Protein concentration by micro-volume ultrafiltration. A: application of sample (FITC-albumin) droplet; B: droplet on membrane surface just after application; C: protein concentrate retained on membrane surface; D: recovery of protein concentrate by pipetting.

Table 3 Concentration of BSA solution by micro-volume ultrafiltration (BSA concentration in feed: 1 mg/mL; buffer: 50 mM sodium phosphate, pH 7.0; membrane: 10 kDa MWCO PES coated on the permeate side with 200 kDa PEO; feed droplet volume: 20 µL)

Time (min)	Mass of retentate (mg)	Volume of retentate (mL)	Concentration of retentate (mg/mL)	Concentration factor (-)	Recovery (%)
0	20	0.020	1.00		
6	8.1	0.008	2.38	2.38	95.2
12	2.1	0.002	9.05	9.05	90.5

The sequence of events in a typical protein buffer exchange/desalting/purification experiment is shown in Figure 6. Figure 6A shows a droplet of FITC-albumin being applied on the surface of a PEO coated PES membrane; Figure 6B shows the droplet immediately after application; Figure 6C show the retentate left behind on the membrane surface; Figure 6D shows the retentate being re-dispersed in buffer; Figure 6E shows the FITC-albumin retained on the membrane surface being recovered. Table 4 summarizes the experimental results obtained from experiments carried out to desalt 5 µL of 5 mg/mL BSA solution using the PEO coated PES membrane. The feed solution was prepared in 50 mM sodium phosphate buffer (pH 7.0) containing 1 M sodium chloride. The objective of the experiments was to remove sodium chloride from the protein solution. The extent of salt removal was assessed by measuring the refractive index of the material recovered from the membrane. The high refractive index of the feed solution (see Table 4) was primarily due to the presence of sodium chloride. A comparison with protein-free 50 mM sodium phosphate buffer (pH 7.0) + 1 M sodium chloride solution shows that the presence of BSA (5 mg/mL) had a relatively smaller contribution to the refractive index. The semi-dry residue obtained after the ultrafiltration of 5 µL of feed solution was re-dispersed in 5 µL of 50 mM sodium phosphate buffer (pH 7.0). The recovered material showed a much lower refractive index than the feed solution indicating that some of the sodium chloride had already been removed. The material recovered after one and two desalting steps showed the same refractive index indicating that a single desalting step was sufficient for removing the sodium chloride almost completely. This was verified by comparison of refractive index with 5 mg/mL BSA solution prepared in 50 mM sodium phosphate buffer (pH 7.0).

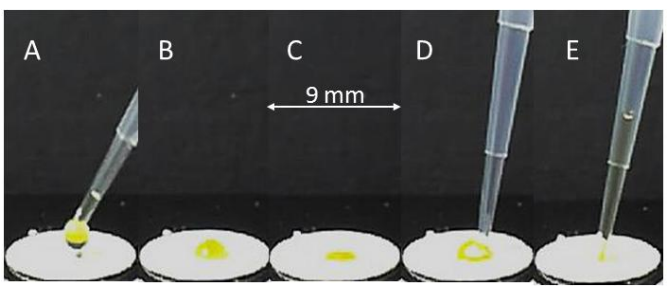

Fig 6 Desalting/buffer exchange of protein (FITC-albumin) by micro-volume ultrafiltration. A: application of sample droplet; B: droplet after application; C: retentate after ultrafiltration; D: re-dispersion of retained protein in new buffer; E: recovery of desalted/buffer exchanged protein by pipetting.

Table 4 Desalting of BSA solution by micro-volume ultrafiltration (BSA concentration in feed: 5 mg/mL; feed buffer: 50 mM sodium phosphate, pH 7.0 + 1 M sodium chloride; desalting buffer: 50 mM sodium phosphate, pH 7.0; membrane: 10 kDa MWCO PES coated on the permeate side with 200 kDa PEO; feed droplet volume: 5 µL; desalting buffer droplet volume: 5 µL)

Material	Refractive index
50 mM sodium phosphate buffer	1.3375
50 mM sodium phosphate buffer + 1 M sodium chloride	1.3465
5 mg/mL BSA in 50 mM sodium phosphate buffer	1.3385
Feed	1.3475
Re-dispersed material after initial ultrafiltration	1.3400
Re-dispersed material after first desalting step	1.3380
Re-dispersed material after second desalting step	1.3380

The feasibility of carrying out size-based protein purification using the micro-volume ultrafiltration technique was demonstrated using BSA (MW 67 kDa) as the model target protein and lysozyme (MW 14.1 kDa) as the low molecular impurity protein. The objective in these experiments was to retain BSA and remove lysozyme in the permeate. The feed solution consisted of 0.5 mg/mL each of BSA and lysozyme in 20 mM sodium phosphate buffer (pH 7.0). A feed droplet of 10 µL volume was deposited on a single disc of PEO coated PES membrane. The dry residue left behind on the membrane surface was re-dispersed 10 µL of sodium phosphate buffer and collected. The feed and retentate samples were analysed by SDS-PAGE (see Figure 7). The feed sample showed two bands corresponding to BSA and lysozyme, while the retentate sample contained mainly BSA and very small amount of residual lysozyme as evident from a very faint band. These results are particularly very encouraging as lysozyme could be almost completely removed even without using any wash buffer. A comparison of the BSA bands in the feed and retentate lanes indicates that protein recovery was very high.

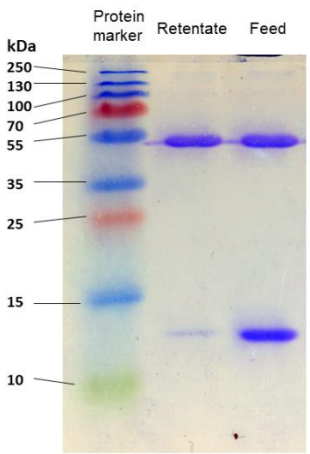

Fig 7 SDS-PAGE (12.5%, non-reducing) gel obtained with samples from size-based BSA-lysozyme separation experiments carried out using PEO coated PES membrane.

The surface of the skin layer of most polymeric ultrafiltration membranes such as that of the PES membrane used in the current study are moderately hydrophobic. In the experiments carried out in the current study, this prevented the applied sample droplet from spreading on the membrane surface, effectively localizing it, making it easy to recover the protein retentate by pipetting. This also allowed easy re-dispersion of protein residue on the membrane surface in buffer in desalting and buffer exchange processes. Moreover, if required, several droplets could be processed in close proximity of each other with minimal interference or cross-contamination between samples. To verify this, hIgG1-CD4 monoclonal antibody and BSA solutions were ultrafiltered by applying them as droplets very close to each other on a single disc of PEO coated PES membrane (see Figure 8A). In this experiment, the sample droplets were each of 2 µL volume, the centre of the droplets were 2.4 mm away, and the periphery of the droplets were separated by just 1 mm. As soon as the samples were ultrafiltered, the respective residues were re-dispersed in 2 µL buffer each, and collected for analysis by SDS-PAGE. Figure 8B shows the stained SDS-PAGE (non-reducing) gel obtained with the feed hIgG1-CD4 and BSA solution as well as the material recovered after ultrafiltration. Quite clearly there was no cross-contamination. The band intensities also indicate that the recovery of protein was high in each case. Based on the above, an alternative format for high throughput ultrafiltration is shown in Figure 9. Instead of using individual membrane discs for the different protein samples, these could all be processed on a continuous sheet of membrane. The osmolyte coating is applied in the form of spots on the permeate side of the membrane, corresponding to the locations where the samples are to be applied as shown in the figure. Alternately, the osmolyte could simply be coated uniformly on the permeate side of the membrane. Based on the sample volume (i.e. 2 µL) and application layout used (i.e. 2.4 mm spacing) in the experiment described in Figure 8A, 1820 samples could potentially be processed in parallel in a 85 mm × 127 mm rectangular membrane sheet, which has the same dimension as a standard 96 well plate. With hydrophilic membranes where sample spreading could be an issue, the membrane format shown in Figure 10 could be used. Here the membrane discs are glued on the underside of a perforated plate and this creates "well-like" slots within which the samples could be added and processed, without them spreading and interfering and cross-contaminating neighbouring samples.

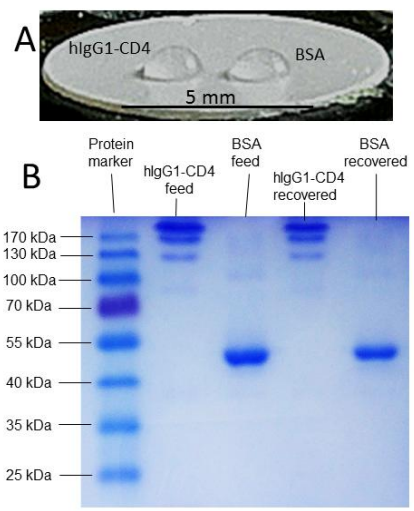

Fig 8 A: Micro-volume ultrafiltration of monoclonal antibody (hIgG1-CD4) and BSA solution sample droplets in close proximity (sample droplet volume: 2 µL each; separation of centre of the droplets: 2.4 mm, separation of droplet periphery: 1 mm). B: SDS-PAGE (10%, non-reducing) gel obtained with the feed hIgG1-CD4 and BSA solutions as well as the material recovered after micro-volume ultrafiltration.

Table 5 shows the time taken for ultrafiltration of 10 µL of FITC-albumin solution (2 mg/mL) using PES membranes coated with a combination of PEO and PAA combination. As in the experiments carried out with PES, and PEO coated PES membranes, the ultrafiltration time was defined as that required for forming a semi-dry protein residue on the membrane surface. Quite clearly, the ultrafiltration time was significantly reduced by the additional PAA coating (see Tables 2 and 5). Figure 11 shows the mechanism by which water is removed from the sample droplet through the membrane to the permeate side. In a PEO coated PES membrane, the osmolyte solution becomes progressively dilute which results in decrease in osmotic draw with time. The presence of the additional PAA coating reduces such primary osmolyte dilution. As the water reaches the PAA layer, it is rapidly absorbed and the osmotic draw of the primary osmolyte is thereby sustained. While the ultrafiltration time with the PEO and PAA combination coated PES membrane was lower, it varied quite significantly as evident from the magnitude of error range in ultrafiltration time shown in Table 5. This was because the exact quantity of PAA in the coating and thereby its thickness was hard to control using the membrane fabricating technique used in the current study. Therefore, while these preliminary results obtained with the PEO and PAA combination coated PES membrane are very promising, the coating method clearly needs further improvement and this will be addressed in future studies.

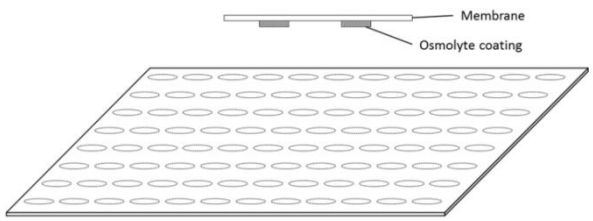

Fig 9 Alternative format for high-throughput micro-volume ultrafiltration with localized osmolyte coating on continuous membrane sheet.

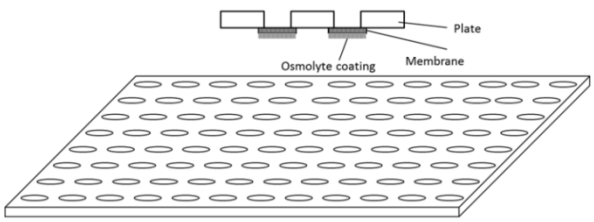

Fig 10 Alternative format for high-throughput micro-volume ultrafiltration with well-like features.

The results discussed above clearly demonstrate the suitability of the micro-volume ultrafiltration technique for concentrating, desalting and buffer exchange of protein samples in the sub 10 µL range. Moreover, the only type of material handling involved in this technique is deposition or collection of droplets by pipetting. Therefore automated 96 well and higher format pipetting systems[27] that are widely available could be used for carrying out micro-volume ultrafiltration in an automated high-through mode. It could also potentially be used for a range of other applications such as size-based purification of protein samples (i.e. removal of low molecular weight impurities and contaminants such as peptide and DNA fragments), clean-up of PCR samples, protein crystallization, and indeed any application where macromolecules are required to be separated from small molecules, e.g. separation of unreacted monomers from polymers. One of the interesting observations from this current study is that if protein is allowed to remain on the membrane surface after ultrafiltration, it forms a defined dry deposit, which could subsequently be re-dispersed in buffer and recovered as a protein solution. This opens up the possibility of using this technique for drying proteins or for creating protein microarrays[28] on a membrane, for micro-scale protein storage and subsequent recovery, all compatible with high-throughput processing. Overall, there is significant potential for follow-up work on the micro-volume ultrafiltration technique discussed in this paper.

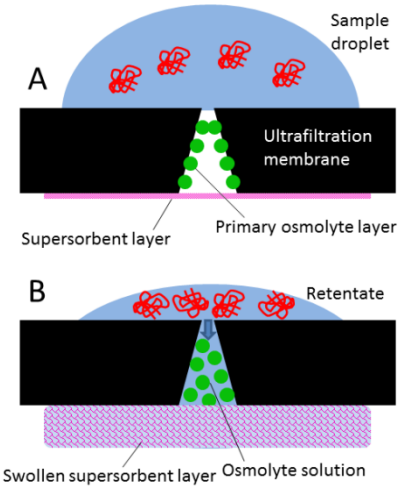

Fig 11 Micro-volume ultrafiltration using combination of osmolyte and supersorbent.

Table 5 BSA-FITC micro-volume ultrafiltration using PEO + PAA coated membranes (BSA-FITC concentration in feed: 2 mg/mL; feed buffer: 50 mM sodium phosphate, pH 7.0; sample droplet volume: 10 µL) (data obtained from experiments carried out in triplicate)

Membrane	Time (min)
10 kDa MWCO PES coated on the permeate side with 200 kDa PEO and PAA	7.3±1.4

Conclusion

The micro-volume ultrafiltration technique discussed in this paper is simple and inexpensive, and suitable for concentrating, desalting and buffer exchanging sub 10 µL volume protein samples, in a high-throughput mode. It does not need any ancillary equipment such centrifuges or devices for applying suction. With the poly (ethylene oxide) (or PEO) coated membrane, the liquid was drawn into and then through the membrane from the applied sample droplet by a combination of capillary action, facilitated pore wall wetting and osmotic draw. The additional poly (acrylic acid) (or PAA) layer in the PEO and PAA combination coated membrane enhanced the ultrafiltration rate by soaking up the drawn water, thereby sustaining the osmotic pressure difference between the sample and the osmolyte solution. The mild hydrophobicity of the surface of the polyether ultrafiltration membrane used in the current study prevented the applied sample droplet from spreading, thereby making it easy to process and recover. In a

protein concentration process, the concentrated protein retentate was directly recovered from the membrane surface by pipetting. In a protein desalting or buffer exchange experiment, the retentate was topped-up with buffer as many times required with buffer, and eventually re-dispersed in buffer by repeated aspiration and dispensing before recovery from the membrane surface. Typical protein recoveries were greater than 90%. Different samples could be processed in close proximity without cross-contamination.

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Notes and references

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