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Nanofiber adsorbents for high productivity continuous downstream processing

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ABSTRACT

An ever increasing focus is being placed on the manufacturing costs of biotherapeutics. The drive towards continuous processing offers one opportunity to address these costs through the advantages it offers. Continuous operation presents opportunities for real-time process monitoring and automated control with potential benefits including predictable product specification, reduced labour costs, and integration with other continuous processes. Specifically to chromatographic operations continuous processing presents an opportunity to use expensive media more efficiently while reducing their size and therefore cost. Here for the first time we show how a new adsorbent material (cellulosic nanofibers) having advantageous convective mass transfer properties can be combined with a high frequency simulated moving bed (SMB) design to provide superior productivity in a simple bioseparation. Electrospun polymeric nanofiber adsorbents offer an alternative ligand support surface for bioseparations. Their non-woven fiber structure with diameters in the sub-micron range creates a remarkably high surface area material that allows for rapid convective flow operations. A proof of concept study demonstrated the performance of an anion exchange nanofiber adsorbent based on criteria including flow and mass transfer properties, binding capacity, reproducibility and life-cycle performance. Binding capacities of the DEAE adsorbents were demonstrated to be 10 mg/mL, this is indeed only a fraction of what is achievable from porous bead resins but in combination with a very high flowrate, the productivity of the nanofiber system is shown to be significant. Suitable packing into a flow distribution device has allowed for reproducible bind-elute operations at flowrates of 2,400 cm/h, many times greater than those used in typical beaded systems. These characteristics make them ideal candidates for operation in continuous chromatography systems. A SMB system was developed and optimised to demonstrate the productivity of nanofiber adsorbents through rapid bind-elute cycle times of 7 s which resulted in a 15-fold increase in productivity compared with packed bed resins. Reproducible performance of BSA purification was demonstrated using a 2-component protein solution of BSA and cytochrome c. The SMB system exploits the advantageous convective mass transfer properties of nanofiber adsorbents to provide productivities much greater than those achievable with conventional chromatography media.

1. Introduction

The biotechnology market is the fastest growing sector within the world pharmaceutical market accounting for 20% ($153bn) of all market sales in 2012. This growth from 10% of the market share in 2002 is set to grow to 41% between 2012 and 2018 from $153bn to $215bn (Strickland, 2012). There are currently around 200 monoclonal antibodies products on the market and with over 1000 in clinical trials, the need for technological advancement is clear (Rader, 2013). Over the last decade we have seen fermentation titres of biomolecules grow from 0.5 g/L to 50 g/L, and while the downstream purification processes have also received must recent research and development, the scale of improvements have not matched those in the upstream (Lightfoot and Moscariello, 2004; Roque et al., 2004). The heavily relied on bind/elute chromatography unit operations are, in economic terms, the key to advancing downstream processing (Labrou and Clonis, 1994; Lowe, 2001).

While conventional packed bed chromatography has been an extremely powerful separation tool it is ever more apparent that

Abbreviations: DEAE, diethylaminoethyl; SMB, simulated moving bed.

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radically new systems must be employed to bridge the current development gap. One route is to change the current support structures from diffusion limited packed bed adsorbents to convective mass transfer media, such as porous membrane adsorbents and monoliths, and remove the two major associated drawbacks of pressure drop and residence times. Thereby addressing the inefficient use of expensive chromatography resins (Charcosset, 1998; Ghosh, 2002). The development of membrane adsorbent structures that allow for flowrate independent operation offers the advantage of increased throughput but has generally only proven useful at small scale with the exception of polishing applications (Phillips et al., 2005). Issues with membrane adsorbent fouling are common and often limit their employment to late stage polishing chromatography operations when larger particulates have been removed (Weissenborn et al., 1997). There is a trade-off to be made between fouling and capture capacity with regard to adsorbent pore sizes. Small pore sizes are required for good separation with sharp breakthrough curves but block easily, conversely larger pore size adsorbent (10 to >150 μm) may offer better handling of foulants but small target biomolecules may pass through the adsorbent without binding (Sarfert and Etzel, 1997; Tejeda et al., 1999; Tennikov et al., 1998). Previous studies have shown that membrane chromatography is a potentially viable alternative in flowthrough mode (Kalbfuss et al., 2007; Zhou and Tressel, 2006). Another focus has been the development of monolith structures which have proved to offer good separation for large biomolecules such as plasmids and viruses due to the relatively large surface pores that they have (Hahn et al., 2002; Jungbauer, 2005).

Recent advances in electrospun nanofiber adsorbents for bioseparation offer improved properties over cast porous membranes, such as a higher surface area and porosity (Hardick et al., 2013; Ma et al., 2009; Zhang et al., 2008). They are fabricated by electrospinning, where a high voltage is applied to a polymer-solvent solution causing surface repulsion which stretches the polymer into nanometre scale fibers before deposition in a non-woven random fashion. Using a randomly deposited fiber mat in liquid filtration systems encourages impeded flow and reduces channelling. For efficient utilisation of the binding surface area the inlet flow must have even dispersion and the pore size distribution must be small to minimize any channelling (Shi et al., 2005). Previously, diethylaminoethyl (DEAE) nanofiber adsorbents have shown an increase in productivity over membrane and packed bed media (Hardick et al., 2013). Nanofibers also have a larger surface area than monoliths in relation to their average surface pore size; this makes them more suited to monoclonal antibody products (Bilad et al., 2011; Lv et al., 2008). Moreover, the breakthrough of protein loaded onto nanofiber adsorbents has been shown to completely saturate the adsorbent at a considerably faster rate than comparable porous membranes (Schneiderman et al., 2011).

Another route for development of improved efficiency is a move into continuous processing. The drive towards continuous processing has been seen in many other industries due to the advantages it expresses. Continuous operation presents opportunities for real-time process monitoring and automated control with potential benefits including predictable product specification, reduced labour costs, and integration with other continuous processes. Little in the way of true moving bed chromatography operation has been developed. Niven and Scurlock (1993) have demonstrated the idea of a moving belt system which enabled a feed stream to be continuously loaded while product was recovered simultaneously. This was brought about by switching the mobile and stationary phases, so that the solid adsorbent phase was moving through a stationary media phase, or rather a number of stationary media phases to allow for loading, washing, elution, cleaning-in-place, and regeneration. Simulated moving bed (SMB) chromatography is currently the most common continuous chromatographic technique used. SMB is based on the flow of a media mobile phase moving counter-current to a constant flow of a solid stationary phase created by switching the position of a series of packed beds. Counter-current flow enhances the potential for the separation making the process more efficient.

The technology has been in use for many years in the chemical industry, originally developed for difficult petrochemical separations (Ruthven and Ching, 1989). Later its use in the pharmaceutical industry quickly grew due to its strong ability to perform chiral separations with the first US Food and Drug Administration's approved drug manufactured by SMB technology reaching the market in 2002 with Lexapro (Schulte and Strube, 2001). Traditionally the powers of SMB to carry out separation based on the different moieties of complex components have been focused on systems that yielded poor productivity using batch chromatography. Today however more focus is being placed on bind/elute chromatographic processes in an effort to improve the utilisation of expensive adsorptive resins and reduce the large volumes of buffers used at large scale production (Guest, 1997; Nicoud and Majors, 2000). As described in Fig. 1, SMB chromatography operates by employing three or more fixed adsorbent substrates, such as packed bed columns or membranes, with buffer and feed streams flowing into the system continuously. A counter-current solid substrate is simulated by switching various valve inlet ports periodically. This leads to a greater utilisation of the adsorbent performing the desired separation of the feed stream yielding a continuous product stream by switching outlet ports (Aumann and Morbidelli, 2007; Schulte et al., 2007; Ströhlein et al., 2007). Recently continuous downstream processing options are gaining further attention from industry which suggests that the adoption of such technologies is likely in the near future (Bisschops and Brower, 2013). Even though SMB systems have been used for the production of marketed pharmaceuticals it has not been widely adopted in bioprocessing (Pollock et al., 2013). This is mainly due to the risk associated with the change from tried and tested unit operations for new ones which bring with them a new range of validation criteria, and unless there is a significant advantage in using such a system its adoption will remain limited.

In this study, we propose the combination to exploit the favourable properties of an electrospun nanofiber adsorbent in a continuous protein purification SMB system. The high surface area material provides easily accessible adsorption sites with enhanced flow properties that allow for higher flowrate operations without losing capacity or incurring large pressure drops. Capitalising on rapid bind-elute cycles via convective mass transfer and full utilisation of the adsorbent media, we evaluate the productivities in a specially designed SMB system.

2. Experimental

2.1. Adsorbent preparation

The electrospun nanofiber adsorbent developed in this study is a cellulosic material chosen due to its wide availability, low cost, and current use in bioprocess purification membranes. Polymeric cellulose is not soluble in common solvents which makes electrospinning it problematic. To overcome this it has become common place to use cellulose derivatives in fabrication processes which can then be treated post-fabrication to form regenerated cellulose. A 0.20 g/mL solution of cellulose acetate (Sigma–Aldrich, Dorset, UK) with a relative molecular mass of 29,000 g/mol, was dissolved in a ratio of 2:2:1 of acetone:dimethylformamide:ethanol (Sigma–Aldrich). The electrospinning process was carried out in a Climate Zone climate control cabinet (a1-safetech Luton, UK) to allow temperature and humidity control of the ambient conditions.

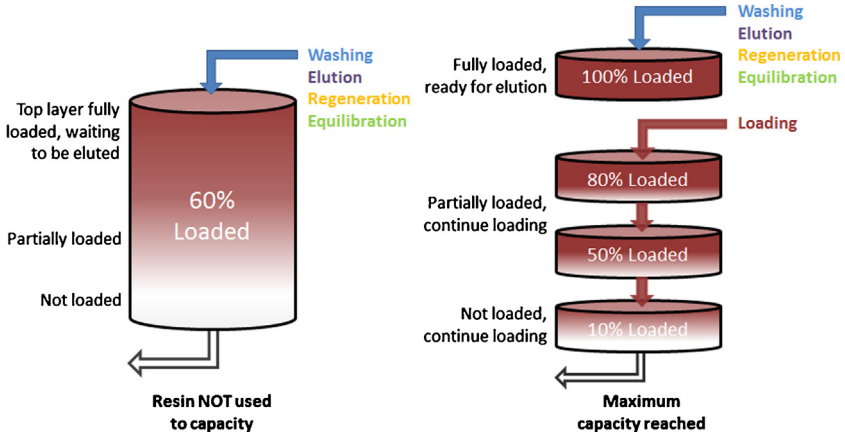

Fig. 1. Utilisations of conventional batch chromatography, left, and simulated moving bed chromatography, right, showing how maximum capacity of columns can be achieved.

Controlling the atmospheric conditions during the electrospinning process will give a consistent nanofiber diameter and deposition, which will ensure suitable distribution properties. Optimized conditions from a previous study were used to produce cellulose acetate nanofibers with a low distribution of fiber diameters (Hardick et al., 2011). Once electrospun the nanofibers were cut into multiple 25 mm diameter discs and treated in 0.1 M NaOH (Sigma-Aldrich) in a 2:1 water:ethanol ratio to obtain regenerated cellulose discs. Discs were packed into a bespoke adsorbent holder designed specifically to support nanofiber adsorbents of varying bed heights while optimising flow distribution and accommodating high flowrates. Anion exchange surface functionality in the form of DEAE groups was then carried out in situ using previously determined chemistry (Hardick et al., 2013). Briefly, a 15% 2-(diethylamino) ethyl chloride hydrochloride (Sigma–Aldrich) solution was used to treat regenerated cellulose discs for 15 min followed by treatment with aqueous 0.5 M NaOH. The resulting bed height of 0.3 mm gives an adsorbent volume of ~0.15 mL, which was used for all experimentation.

2.2. SMB design and operation

A simulated moving bed system was designed and built using a series of Burkert solenoid valves (Bürkert Fluid Control Systems, Stroud, UK), 1/16″ Peek tubing, Peek connectors, and three Dionex P580 P HPLC pumps (Dionex Softron GmbH, Germering, Germany). UV sensors were placed on the exit of each of the three adsorbent holders which were connected to a National Instruments analogue input module (Newbury, UK) to record the UV absorbance at 280 nm. Three National Instrument digital output modules were used to control the valve positions with National Instruments Labview 2010 software (Newbury, UK), sequence the control and compute the analogue input signals. Fig. 2 shows examples phases of operation including the three columns, UV sensors, pumps, 18 valves, and buffer lines.

DEAE nanofiber adsorbent cartridges were used in the custom adsorbent holders and equated to a bed volume of 0.45 mL. The cartridges were fitted in-line to the SMB rig. A two-component protein solution consisting of BSA 1 mg/mL (Fraction V, >96%, ~66 kDa, Sigma–Aldrich) and 0.25 mg/mL cytochrome c (Equine Heart, ≥90%, ~12 kDa Merck Serono Ltd. Middlesex, UK) was used to assess protein separation performance of the bespoke SMB system. The binding and wash buffer used was 20 mM Bis–Tris, pH 5.3 and the elution buffer was identical plus 0.3 M NaCl. A mixture of BSA and cytochrome c was chosen due to their different isoelectric points and therefore suitability for separation by ion-exchange chromatography. Cytochrome c has a p*I* of 10.0 while BSA has a p*I* of 4.7 in water at 25 °C. This means that in a Bis–Tris buffer solution at pH 5.3 the cytochrome c will have a net positive charge and will not bind to the weak anion exchange surface of the DEAE adsorbent. In contrast, at this pH above the p*I* of BSA, the protein will have a net negative surface charge and therefore will bind to the DEAE adsorbent. As the salt concentration is increased during elution the interaction between the negative surface charge of the BSA and the anion exchanger is out competed by the salt ions and so the BSA is removed from the adsorbent and collected. While this system is being used here to show proof of concept for a bind-elute application it is worth noting that the complexity of feedstreams in an industrial setting would make such a separation more challenging.

Fig. 3 illustrates the phase of operation that each adsorbent column is in at a particular point in time. This is translated into an expected UV absorbance trace following the exit of each column to demonstrate the continual loading and elution of target molecule. This process can only be optimised within the limits of the SMB system which gives the user the opportunity to change the phase flowrates and valve switching times to direct the phase media accordingly. Once the flowrate and valve switching times were optimised the continuous purification was left to run for a period of 10 min to establish the productivity of each run. Productivity was determined by dividing the summed DBC of the columns by the time taken for a complete cycle of the 3 column SMB system. DBC's for the nanofibers and membranes were established by operating the columns in a conventional manner at the same flowrate that was ultimately used in the SMB process. The usual approach of 10% breakthrough and peak integration using unicorn software was carried out. In the case of the bead resin the supplier reported literature value was used. The SMB operation was repeated using Sartobind IEXD membrane (Sartorius Stedim, Epsom, UK) at an equivalent bed volume.

2.3. Batch protein separation, reproducibility and lifecycle performance

Batch experiments were completed using an AKTA Basic (GE Healthcare Life Sciences, Buckinghamshire, UK) with online measurement of UV absorbance (280 nm), pH, and conductivity. Batch separation was used to evaluate the dynamic binding capacities of the DEAE nanofiber adsorbent and Sartobind IEXD membrane. A DEAE nanofiber cartridge was equilibrated with 5 mL 20 mM Bis–Tris, pH 5.3 wash buffer at a rate of 480 cm/h. A 0.5 mL injection of the 2-component protein solution consisting of 1 mg/mL BSA and 0.25 mg/mL cytochrome c was loaded and 5 mL wash buffer was

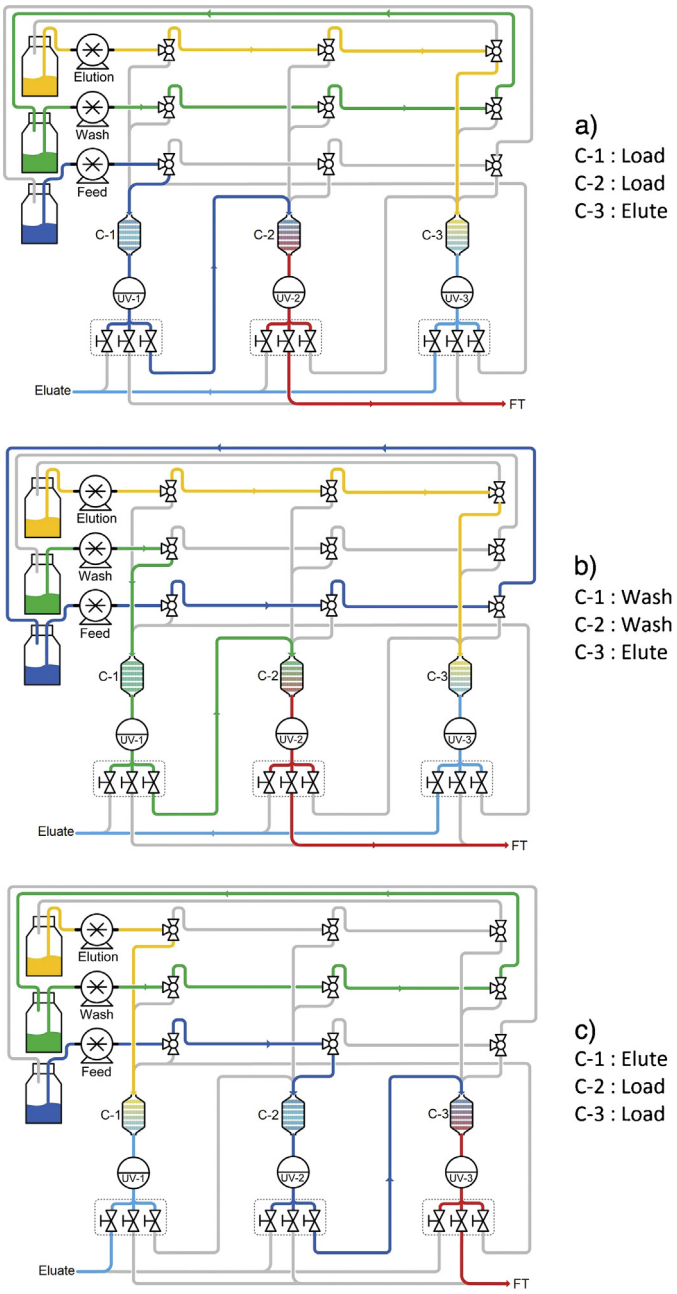

Fig. 2. SMB system design showing different phases of operation. (A) Column 1 (C-1) & C-2 load phase, C-3 elute & collect phase. (B) C-1 & C-2 wash phase, C-3 elute & collect phase. (C) C-1 elute & collect phase, C-2 & C-3 load phase.

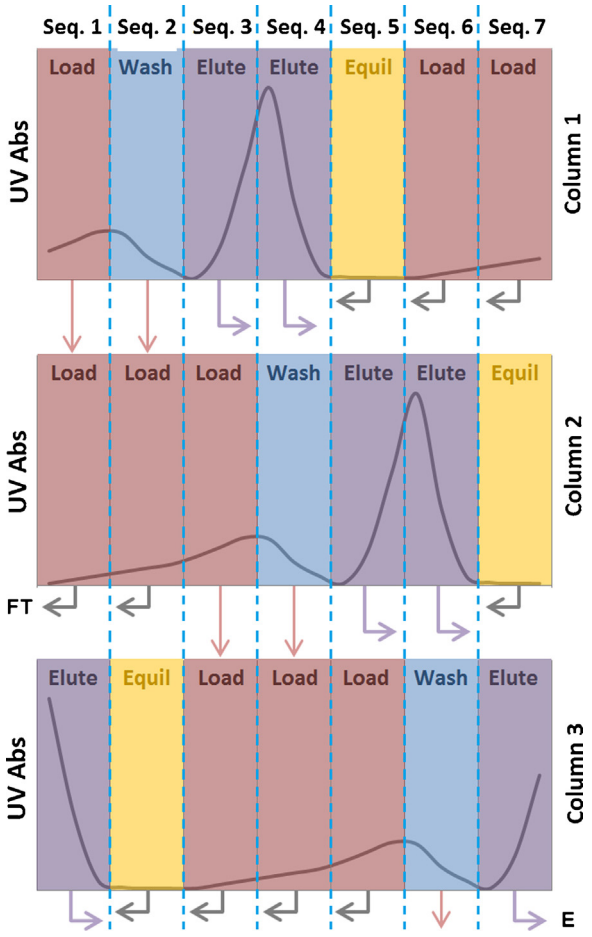

Fig. 3. SMB phase operation with UV absorbance traces following the exit of each column. This diagram illustrates the operation of the simulated moving bed system highlighting the media phase at each column for a given point in the sequence. At the exit of each column is a UV 280 nm sensor which creates an analogue voltage signal that is continuously read by LabVIEW software. An example of these UV traces is given in the diagram and follows the sequence given. E refers to the eluate and FT refers to the flowthrough.

then passed through the adsorbent before 5 mL 0.3 M NaCl 20 mM Bis–Tris pH 5.3 elution buffer was introduced. This was repeated 20 times with 2 mL fractions being collected during loading and elution. The same protocol was run with an equivalent bed volume of Sartobind IEXD membrane. As a control non-derivatised cellulose nanofiber adsorbents were investigated and showed no binding.

The reproducibility characteristics of the nanofiber adsorbents were investigated in two parts. Firstly, 10 different DEAE nanofiber adsorbents of equal volume were fabricated and performance tested to establish variants in performance caused by fabrication. The 2-component separation was carried out as described above with the same operating conditions. The second part of the reproducibility study was to measure the performance of the adsorbents before and after employment in the SMB system for 30 min. This was to show reproducibility with regard to the lifetime of the adsorbents and was carried out as above in the AKTA Basic system. The lifecycle performance of the nanofiber adsorbents was investigated using a Hitachi TM-1000 Tabletop scanning electron microscope (Hitachi High-Technologies Europe Gmbh) to analyse the surface morphology of the nanofibers and observe any fouling.

2.4. Protein analysis

SDS-Page precast 12-well 10% Gels (Expedeon Ltd. Cambridgeshire, UK) were used to analyse the components in product and waste streams. Samples were diluted to similar concentrations and denatured with Laemmli 2×, 1 part sample to 1 part denaturing agent with a total volume of 20 μL. The samples were then denatured at 95 °C for 11 min. The SDS plate was rinsed with DI water and secured into the running cassette. The tank was then filled with running buffer (RunBlue SDS Reducing Run Buffer) until full. The denatured samples were then added to the wells before the electrodes were connected and turned on to 110 V for 90 min. After this the cassette was removed and split to release the gel which was rinsed with DI water and left in Coomassie Brilliant Blue staining agent for 2 h. The gel was de-stained overnight in 30% methanol + 10% acetic acid before being rinsed with DI water.

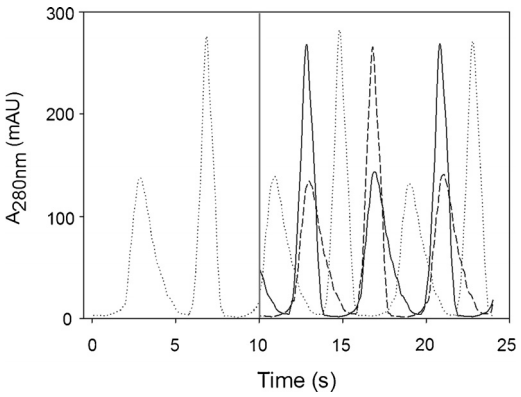

Fig. 4. Representative chromatogram data from the SMB operation of a 2-component separation. The UV absorbance traces were a snapshot for a 25 second time period taken from the three different adsorbent columns (Column 1 (dotted) Column 2 (dashed) Column 3 (solid)) during one SMB operation. Column 2 and 3 are removed from the first 10 s to show the rapid cycle of the process. The high level of consistency observed highlights the suitability of nanofiber adsorbents for operation in continuous processing applications.

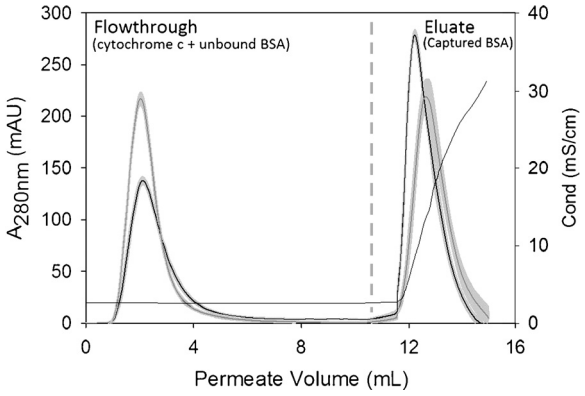

Fig. 5. Chromatogram of 2-component separation showing average results for DEAE nanofiber adsorbent (dark grey) and Sartobind IEXD (light grey). ±1 standard deviation of the sample population is shown by lighter shaded area around the main curve of the same colour. This demonstrates that for the conditions chosen (see Section 2.3) the nanofiber adsorbent captured and eluted more of the target molecule (BSA) from the 2-component mixture. The tighter shaded region also indicates a more consistent performance in separation. The average conductivity had been plotted on the secondary *Y* axis. In each case the adsorbent tested had a bed height of 0.3 mm and column volume of ~0.15 mL.

3. Results and discussion

3.1. SMB and batch operations

The SMB system designed for this study relies on a series of valves to switch at given time points to direct the flow of the different mobile phases. In order to optimise the performance of the system, and therefore productivity, the 18 valves must direct the different phases to different locations at exactly the right time due to the high flowrates employed which demanded ±0.05 s accuracy when switching the direction of flow. The chromatogram in Fig. 4 comes from data collected by the three SMB UV sensors attached at the column outlet. The signal that they created was fed back into the NI analogue input module and recorded by a Labview program that was specifically written for this purpose. The traces show how all three adsorbent columns operate similarly and how the different phases are running through each column at a particular time. We observed the flowthrough as the shorter peak containing unbound proteins of the 2-component solution (cytochrome c + BSA) and the elution being the taller peak containing our captured target molecule, BSA. One bind-elute cycle for a single column was completed in 7 s. The similar peak heights and shapes suggest repeatable cycles were completed, indicating the product stream was of a consistent protein concentration and capacity of the adsorbent was retained.

An AKTA system was used to investigate the bind-elute profiles of the DEAE nanofiber adsorbent and a commercial Sartobind IEXD membrane. From this we quantified the dynamic binding capacity and calculated the separation performance of SMB operation. Fig. 5 shows the flowthrough of cytochrome c + unbound BSA as the first peak during loading of the 2-component mixture and the elution as the second peak. The graphs show a combination of 12 equivalent runs for each adsorbent type at a flowrate of 480 cm/h. For the conditions chosen, the nanofiber adsorbents performed favourably over the Sartobind IEXD membrane for an equivalent volume of adsorbent. The nanofiber adsorbent captures and elutes 99% of the BSA loaded, whereas the membrane only captures 70%. Over the 12 runs for each adsorbent the standard deviation was calculated. The nanofiber adsorbent operated more consistently, although both types demonstrated high levels of consistency. The rapid convective mass transfer utilised in this study demonstrates the immediate nature of the binding events since contact times of the load and elution fractions were 0.3 s and 1.1 s, respectively. This presents an opportunity for a significant reduction in cycle times compared with packed-bed SMB systems. This study achieved full cycles in 15 column volumes of liquid phase and showed potential for further optimisation which offers advantages when considering a typical 25 column volume process for a packed bed resin (GE Healthcare, 2014a,b).

An SDS Page gel was used to investigate the flowthrough and eluate solutions from the SMB and batch separations. Fig. 6 shows the components of the protein load mixture including BSA aggregate at 132 kDa and BSA whole cell at 66 kDa (well 1), and cytochrome c at 11 kDa (well 2). The presence of an 11 kDa in the flowthrough wells confirms the buffers conditions were suited to

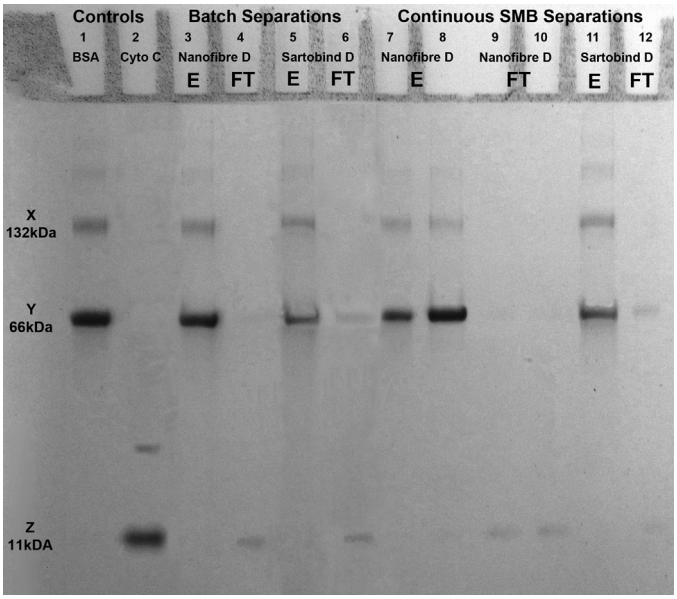

Fig. 6. SDS Page Gel showing denatured components protein samples from the flowthrough and elute stages of nanofiber DEAE and Sartobind IEXD adsorbents during 2-component separation in batch and SMB operations. Components shown in the X band are likely to be BSA aggregates; the Y band indicates whole BSA components while the Z band shows whole cytochrome c components. E refers to the eluate and FT refers to the flowthrough. The gel indicates that the BSA was separated successfully from a 2 component solution (BSA and cytochrome c) under the chosen consitions (see Section 2.3). Moreover nanofiber adsorbents performed preferentially in both the modes of operation, where full utilisation of the media was attained.

Table 1
Purification productivities achieved during SMB operations of nanofiber DEAE and Sartobind IEXD. Operational flowrates were chosen to maintain a standard pressure drop of 0.125 bar across the nanofibers and membranes. The manufacturer advised flowrate was used for the DEAE FF Sepharose resin. Productivity calculations (expressed as Grams of product per Millilitre of adsorbent per hour) were based on the following column dimensions: Porous beaded system 0.7 × 2.5 cm (W × H), other adsorbents 2.5 × 0.204 cm (W × H). Productivity calculations take into account all phases of a standard cycle of operation including regeneration times.

Adsorbent	Dynamic binding capacity (mg/mL)	Operating flowrate		Productivity (g/mL/h)
		(CV/h)	(cm/h)	
Nano-DEAE	10	12000	360	3.92
Sarto-IEXD	6.5	6000	180	1.22
DEAE Sepharose	110	60	150	0.20
Capto DEAE	90	60	600	0.17

the 2-component separation. The Sartobind IEXD membranes did not capture all of the BSA during the loading and can be seen in the flowthrough sample (well 6) on the gel. In contrast the flowthrough sample from the nanofiber adsorbent (well 4) indicates no BSA present. The same is observed in wells 9 and 10 showing the flowthrough from two nanofiber DEAE SMB runs. Wells 7 and 8 show the eluate (product) streams from two nanofiber DEAE SMB runs and indicates the same result as the batch separation, suggesting consistent separation between the two operations (well 3). Wells 11 and 12 show the components of the eluate and flowthrough streams during an SMB run using Sartobind IEXD membranes. The eluate is as expected containing only BSA but the flowthrough appears to contain both cytochrome c and BSA, suggesting that not all the BSA was captured as seen with the batch separation in well 6.

Once optimisation of productivity for the 2-component system was complete we were able to use the SMB system to repeatedly purify 200 mg BSA from the two-component protein mixture in less than 7.5 min at 360 cm/h. Using three columns, each of 0.15 mL adsorbent, an overall system productivity of 1.72 g/h was calculated, relating to an system productivity of 3.92 g (product)/mL (adsorbent)/h (Table 1). Sartobind IEXD membrane loaded into adsorbent holders were tested at a flowrate of 160 cm/h to maintain the same pressure drop as the nanofiber adsorbent of 0.125 bar. Combined with a lower capacity, the productivity of the porous membrane was 1.22 g/mL/h and is considerably lower than that of the nanofiber adsorbent. From the batch operation, the capacity of the Sartobind IEXD membrane was only 70% that of the nanofiber adsorbent under these conditions. The unique non-woven matrix of nanofibers offers a higher surface area and improved flow distribution properties over the membrane and becomes advantageous in SMB operation. These improved characteristics of nanofibers have been previously reported (Hardick et al., 2013; Zhang et al., 2008).

For comparison, manufacturer reported capacity and recommended flowrate values of a 1 mL bed volume packed bed resin DEAE Sepharose (GE Healthcare, 2014a,b) and 10 mL bed volume packed bed resin Capto DEAE (GE Healthcare, 2014a,b) were used to estimate SMB productivity in our system. Unsurprisingly, the diffusion limited resin performs at a much lower productivity than convective media. Porous resins cannot operate at the same high flowrates as the packed beds rely on a much slower flowrate to allow for the diffusional mass transfer of biomolecules to reach the internal functional binding surface and this is one reason why industrial SMB systems have been limited to the chiral separation of small drug molecules. Through the use of nanofiber adsorbents we can utilise the performance advantages of SMB with larger biomolecules as shown by a productivity of over 15 times that of the resin in this study. With even higher operational flowrates achievable as shown in the batch operation for these adsorbents further productivity gains can be expected. One potential application where a technology of this sort may enable new processing strategies is in end-to-end integrated continuous bioprocessing of feed streams from perfusion bioreactors, a strategy which has been reported to offer advantages over traditional bioprocessing approaches (Warikoo et al., 2012). It is worth noting that the small bed height nanofiber adsorbents employed in this study are unlikely to be suited to complex gradient separations due to the very short residence times. In such an instance a packed bed system is likely to offer superior separation performance, though the intension here was to demonstrate proof of concept for bind-elute applications.

3.2. Reproducibility and lifecycle analysis

A key economic criterion of a chromatography adsorbent is how it copes with repeated use. As with any biotechnology process the reproducibility in performance is either expected or required by validation and regulation (Rathore and Sofer, 2005). In terms of the commercial prospects of nanofiber adsorbents any product specification documentation will require rigorous quality assurance testing. Fig. 7a shows chromatograms of 2-component separations by 10 different DEAE nanofiber adsorbents of equal volumes run under the same batch operation conditions to show the reproducible nature of fabrication and how this relates to operation. As well as fabricating an adsorbent in a reproducible manner, it is important to demonstrate that the nanofiber adsorbents can be used many times without degradation to performance over time as this is critical for implementation into SMB operation. Fig. 7b shows chromatograms of 2-component separations by the same DEAE nanofiber adsorbents before and after employment in SMB operation for 30 min (>225 bind-elute cycles). The chromatogram shows no change in binding capacity and shows that separation

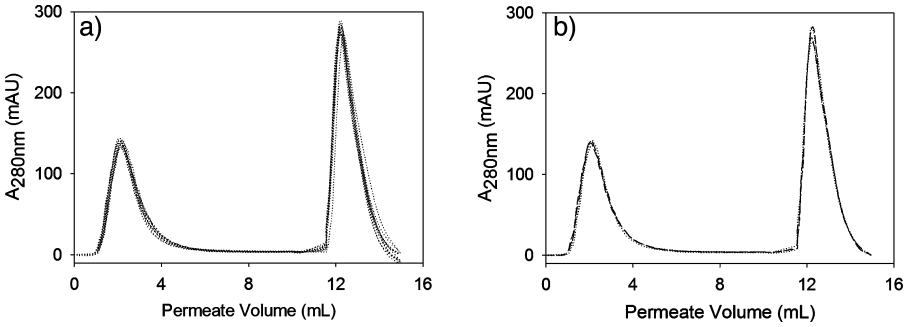

Fig. 7. Representative chromatograms from batch operations to show the reproducibility of DEAE nanofiber adsorbents. (a) Left, Chromatograms showing 2-component separations by 10 different DEAE nanofiber adsorbents demonstrating the reproducibility of fabrication relating to the operation. (b) Right, Chromatogram showing 2-component separations by the same DEAE nanofiber adsorbents before and after employment in SMB operation for 30 min.

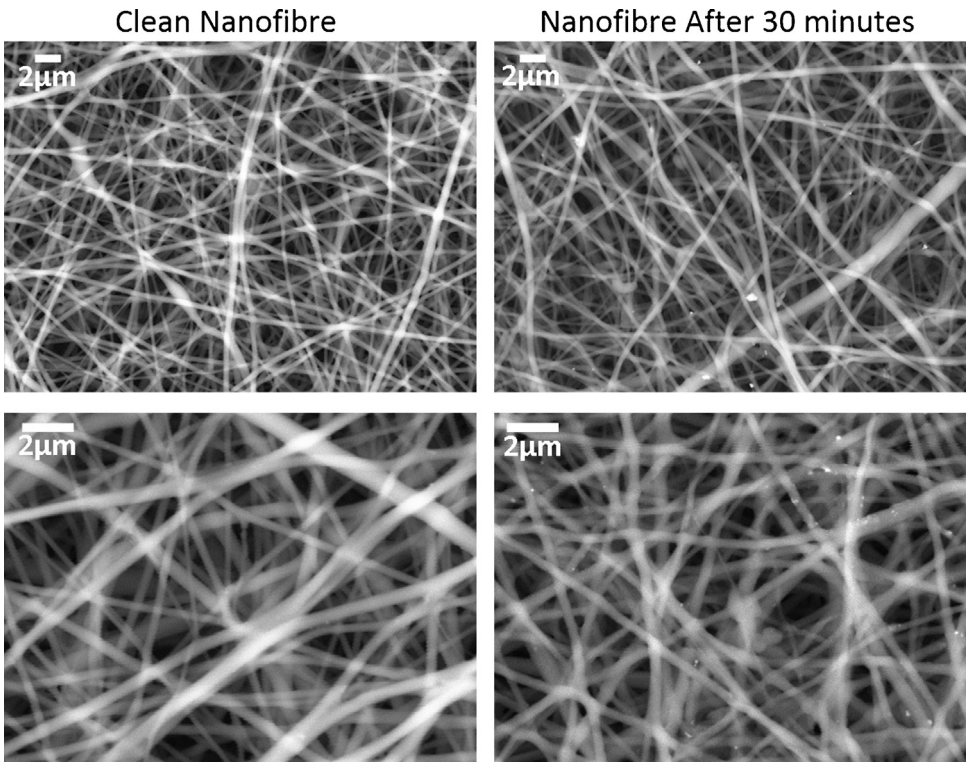

Fig. 8. Scanning electron microscopy images of DEAE nanofiber absorbents after SMB operation. DEAE nanofiber adsorbents remain clean after 30 min (>225 cycles) of SMB operation as suggested by the reproducibility studies. Left, clean unused nanofiber adsorbents at two magnifications. Right, after 30 min of SMB operation. The scale bar indicates 2 μm.

performance was maintained. The reproducibility of separation media is of significant benefit to a process where ligands utilisation accounts for a large proportion of the production costs. Particularly in SMB systems, it is important to confirm that the binding surface remains clean and maintains all of this functional capacity throughout the high number of reuses per time period.

As mentioned earlier the lifetime of chromatographic adsorbents is commonly the most important factor when it comes to establishing feasible process economics. With the results above suggesting reproducible operation of over a sustained period of time we imaged the nanofiber adsorbent using a scanning electron microscope. Fig. 8 shows that the adsorbents are very similar before and after SMB operation suggesting that the interaction between the feed presents few fouling effects. This was expected due to the high purity of the 2-component mixture and the nanofiber adsorbents have been shown to remain clean with complex feeds such as clarified yeast homogenate (Hardick et al., 2013). The highly open porous nature of the nanofiber matrix prevents fouling, which is an issue with porous membrane media. The channels in porous cast membranes tend not to be uniform and when blocked restrict performance. Using convective mass transfer in an SMB system relies on repeated bind-elute cycles and any compromise in capacity and increased back pressure from blocking would result in severely reducing productivity and reproducibility. This would have a knock-on effect in validating such a system with complex feed streams.

3.3. Potential of nanofiber adsorbents in SMB

The productivity that we were able to achieve was limited by the simple design of our SMB system which utilised only three adsorbent modules in sequence and could only be run at 360 cm/h due to the limitations of the HPLC pumps that we had available. Productivity calculations were based on chromatography cycle times for the loading, washing, elution, and regeneration of the adsorbents for set flowrates. Using the productivity ratios that were established in our SMB system, we extrapolated productivities for higher flowrates based on the known pressure drops of each type of adsorbent. In a previous study we have shown in batch operation that nanofiber adsorbents retained a 10 mg/mL dynamic binding capacity, operating at a flowrate of 2400 cm/h with a pressure drop across the adsorbent of less than 0.5 bar (Hardick et al., 2013). The equivalent pressure drop using Sartobind IEXD membranes equated to a flowrate of 1200 cm/h. For the DEAE Sepharose comparison phase lengths were taken from recommended values in literature. Table 2 shows the potential productivities assuming a similar SMB system could operate at these high flowrates. DEAE nanofiber adsorbents show the highest productivity with increases of over 5 times that of Sartobind IEXD and 33 times of DEAE Sepharose. By operating at higher flowrates the productivity factor

Table 2
Comparing potential productivities of different media in SMB operation. The potential productivities of media operating at their maximum established flowrate for a pressure drop of 0.5 bar across the nanofibers and membranes, the manufacturer maximum recommended flowrate was used for the DEAE Sepharose resin. Productivity calculations (expressed as grams of product per millilitre of adsorbent per hour) were based on the following column dimensions: porous beaded system 0.7 × 2.5 cm (W × H), other adsorbents 2.5 × 0.204 cm (W × H). Productivity calculations take into account all phases of a standard cycle of operation including regeneration times.

Adsorbent	Dynamic binding capacity (mg/mL)	Operating flowrate		Calculated productivity (g/mL/h)
		(CV/h)	(cm/h)	
Nano-DEAE	10 (±0.3)	80000	2400	26
Sarto-IEXD	6.5 (±0.71)	40000	1200	5
DEAE Sepharose	110	240	600	0.79
Capto DEAE	90	70	700	0.19

is considerably higher than that shown in our experimental study above. The capacity of Sartobind IEXD was assumed to be the same found during batch experiment but at a flowrate of 1200 cm/h, the porous channel matrix may further restrict mass transfer and reduce capacity, leading to a limited maximum productivity likely lower than we estimated. Combined with the alleviated fouling issue occurring in membranes shown above and in our previous study, the reproducible operation of nanofiber adsorbents are an attractive SMB medium. Understanding that bead resin systems are typically operated with bed heights of 10 cm or above at industrial scale we carried out SMB modelling in the same manner with a 10 mL, 10 cm bed height Tricorn S/100 column. Due to the increased bed height, the cycle times are increased even when operating at 700 cm/h and this in turn reduces the productivity in comparison to the 1 mL Hi-Trap columns. This provides further indication that high flow rate membrane type adsorbers offer significant benefits to operation in a continuous manner.

With continuing development into continuous bioprocessing it can be expected that these technologies will gain more appeal with the industry with their operational advantages which should see a wider uptake in years to come (Cramer and Holstein, 2011). The strict regulation of the pharmaceutical industry has seen batch processes favoured due to more simple traceability. Being a continuous process, SMB still raises many questions and concerns from quality control groups despite having been used in the pharmaceutical industry for decades with approval from the FDA and other regulatory agencies (Mihlbachler and Dapremont, 2005). SMB operations have been investigated and shown to be advantageous though they have yet to become a part of industrial bioprocessing (Sahoo et al., 2009). The complexity of packed bed resin SMB systems raises reliability concerns which could result in excessive downtime and further hinder implementation in industry. For validation, the mechanical and chemical stability of the solid phase adsorbent over long continuous operations must be carefully investigated and understood to maintain the prescribed level of purification (Rathore and Sofer, 2005). Research has been continuing to address the perceived issues of SMB robustness by developing a 'cycle to cycle' controller allowing for an accurate determination of the average concentration of the product stream (Grossmann et al., 2010). The integrity of nanofiber adsorbents used in the system would be an important criterion of the validation process. The appropriate cleaning of a complex valve system such as that used in this study is a vital requirement for validation, and a next generation design might employ the use of disposable valves to facilitate validation. There is a commercial SMB device on the market which employs disposable pinch valves (Bisschops et al., 2009). With increased interest in single-use systems, the possibilities to introduce nanofiber adsorbents into a validated system are attractive to alleviate the limitations seen in other media regarding continuous chromatography operations.

4. Conclusions

We evaluated the performances of novel nanofiber DEAE adsorbents and Sartobind IEXD membranes in an SMB system using 2-component protein separation experiments. The experimental productivity of the nanofiber adsorbent was 3.92 g (product)/millilitre (adsorbent)/hour and is considerably higher compared with those of the membrane and packed bed values estimated from manufacturer recommendations. A full bind/elute cycle for nanofiber D was achievable in 7 s, producing around 3 mg product in our three column SMB system. With established properties, we calculated that a nanofiber adsorbent SMB system can potentially reach a productivity of 26 g/mL/h operating at 2400 cm/h, which increased the productivity factors over the membrane (5 g/mL/h) and packed bed resin (1 g/mL/h). The application for scale up is favourable assuming equivalent performance can be achieved. The DEAE nanofiber adsorbent behaves reproducibly with regard to adsorbent fabrication, capacity performance and cleanliness after repeated use, implying suitable characteristics for implementation into SMB operation.

With advancements in upstream technologies, there is a clear need to develop the purification stages of downstream processing. The advantageous nanofiber adsorbent properties of high surface area, flow distribution and fouling resistance flowrate properties, make it a widely applicable technology as a chromatography medium. Adsorbents offer a compromise between capacity and speed with the aim of increasing productivity through frequent short bind-elute cycles therefore reducing the cost of the single most expensive unit operation in a bioprocess. The authors recognise that this is a simple binary separation and does not give indication as to the nanofiber adsorbent's suitability for complex ion exchange separations of similar components. As is common with membrane adsorbers, where short bed heights are employed the operator is typically limited to impurity capture or bind-elute operations, such as those with affinity ligands. In a future study it would be possible to modify the nanofiber adsorbents into a structure more akin to a packed bed or monolith system with a greater bed height which could then be investigated to see if this offered the efficiency/number of plates for difficult separations which require gradient elution conditions. It is worth noting that from a manufacturing view point, if a simple bind-elute system were employable to give effective separation this would be a more robust processing option over a system which required a more complicated gradient elution. Continuous purification and SMB systems are ideal modes of operation to exploit the operational and convective mass transfer properties of nanofiber adsorbents and maximise purification processes.

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