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Short communication

Microscale stirred-cell filtration for high-throughput evaluation of separation performance

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ABSTRACT

Membrane filtration is a key separations technique in downstream bioprocessing applications: for a commercial bioprocess operation, 10–20 membrane-related steps are typically required. At the laboratory scale, the evaluation of membrane separation performance often involves a 'stirred cell device'; however, this simple tool is poorly suited for conducting high-throughput studies of separation performance. Here, we designed a high-throughput stirred cell (HTSC) device which is ideally suited for conducting optimization studies, especially at the early stages of bioprocess development when small volumes of feed material are available. The HTSC allows for up to six filtration experiments to be run simultaneously with continuous mixing above each membrane facilitated by suspended magnetic stir elements. Using fluorescently-labeled dextrans as model biomolecules and commercial ultrafiltration membranes, it was shown that the HTSC device gave the same separation performance as a traditional stirred cell, but with the added benefits of operating at a faster pace (due to its parallel nature) and requiring nearly an order of magnitude lower sample volumes. Furthermore, the utility of the HTSC was demonstrated by concurrently evaluating the performance of four different ultrafiltration membranes for the same model biomolecule and the membrane selectivity for a mixture of two model bio-macromolecules.

1. Introduction

Membrane filtration processes are abundant in downstream bioprocessing tasks such as buffer exchange, biomolecule concentration, and biomolecule purification; typically 10–20 membrane filtration steps are used in a single biomolecule production process [1]. These processes are widely used in bioengineering applications, with microfiltration (MF) membranes used in cell harvesting and sterile filtration processes, and ultrafiltration (UF) membranes used in protein purification and macromolecule concentration applications [1]. Furthermore, the rapid growth in the biotechnology field has created an increased demand for the efficient purification of bio-macromolecules [2], including a demand for advanced membrane technologies. Given that up to 85% of the bio-macromolecule production costs are associated with downstream bioprocessing applications [3], significant time- and cost-savings could result from more efficient, high-throughput downstream processing.

It is well-known that membrane performance (*e.g.* selectivity, fouling propensity) in a given application is a complex function of a multitude of factors, including the physico-chemical structure of the membrane itself (*e.g.* pore size/structure, material), the feed solution conditions (*e.g.* pH, ionic strength), process operating conditions (*e.g.* flow rate, hydrodynamics), and flow orientation (*e.g* normal versus tangential flow) [4]. While commercial bioprocesses often rely on tangential-flow filtration methods such as in virus and protein purification applications [2], normal-flow (dead-end) filtration also serves key roles in bioseparations. To elucidate the effects of these process factors, a common approach at laboratory scale involves using a 'stirred cell device' operated in a dead-end filtration mode to evaluate membrane performance. These devices are available from various companies and in different capacities; for example, the Amicon® series (MilliporeSigma) of stirred cells is offered in sizes ranging from 3 to 400 mL to accommodate a range of filtration experiment scales. A circular membrane disc is mounted at the base of device and the flow of fluid is normal to the membrane surface, with mixing enabled above the membrane surface using a mounted magnetic stir bar [5]. For over 40 years, these stirred cells have been used in a wide range of bioseparations research applications. For example, one of the earliest reported studies elucidated the transport of lipoproteins through a UF membrane [6]. More recent studies have used stirred cell devices in applications such as exosome isolation [7], plasmid DNA separation [8], the study of biofouling behaviour [9], and the production of Hepatitis C virus-like particles [10].

While the stirred cell is not the only available membrane evaluation tool (tangential flow filtration cassettes and hollow fiber

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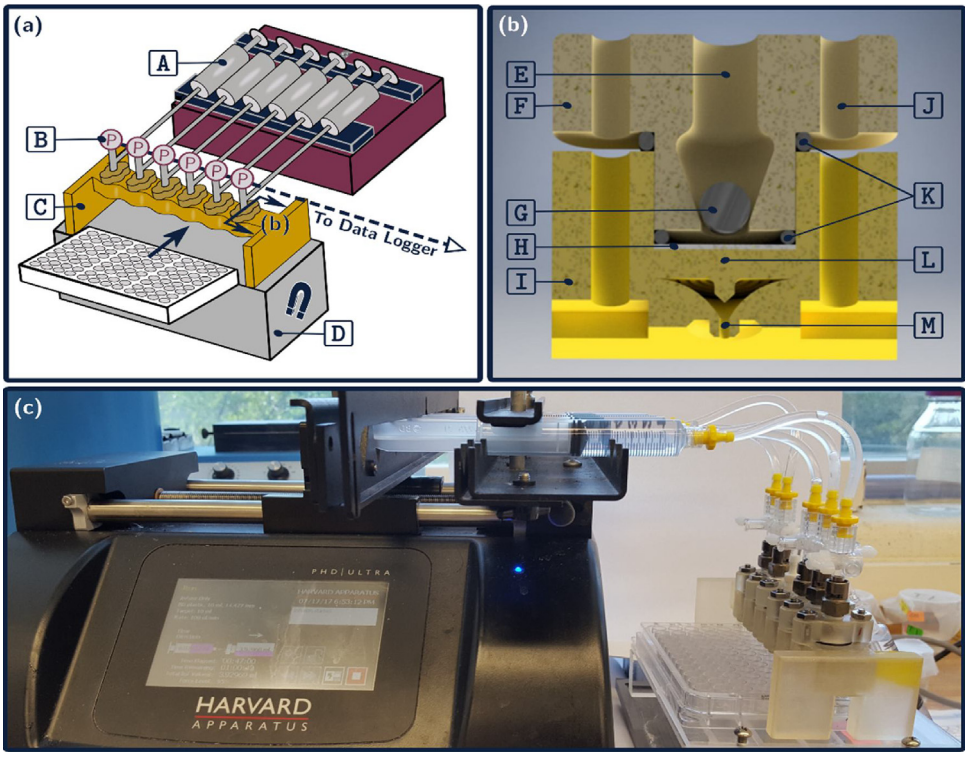

Fig. 1. The high-throughput stirred cell (HTSC) apparatus. **(a)** Schematic of the apparatus, featuring a multi-rack syringe pump [A], pressure transducers (not used in this investigation) [B], the HTSC experimental setup [C], and a magnetic tumble stirrer [D]. **(b)** Half-section cutaway of an individual HTSC cell, showing the cell feed port [E], upper well insert [F], magnetic stir disc [G], membrane disc and stainless steel mesh spacer [H], HTSC chassis [I], 2 mm diameter bores for bolts to fasten the well insert to the chassis [J], O-ring seals [K], porous membrane support [L], and permeate port [M]. **(c)** Image of the HTSC experimental setup. The three-way valve and metal Luer-lock fitting threaded into each cell's feed port facilitates sample collection and infusion.

modules also exist), it is frequently used in evaluating filtration processes at laboratory scale. While it is clearly a useful tool, the laboratory stirred cell device constrains researchers to perform experiments in a sequential fashion, limiting the opportunity to conduct high-throughput studies. There is continued interest in the use of microscale processing techniques to accelerate the development of downstream bioprocessing strategies [11]. For example, we recently developed a high-throughput filtration setup that incorporates mixing above the membrane surface akin to the stirred cell device [12], and demonstrated its use in combination with 'design-of-experiments' strategies to optimize biomolecule separation performance [13]. However, that technique demanded the use of specialized 96-well filter plates and thus was limited to the few commercially-available filter plates with various UF and MF membranes. In this work, we have avoided this limitation by designing a high-throughput stirred cell (HTSC) device that can run up to six filtration experiments simultaneously and can accommodate any flat sheet membrane.

2. Materials and methods

2.1. Dextran solutions

Fluorescein isothiocyanate (FITC)-labeled dextrans with reported molecular weights of 4 kDa and 70 kDa and a tetramethylrhodamine isothiocyanate (TRITC)-labeled dextran with a reported molecular weight range of 65–85 kDa were obtained from Sigma-Aldrich. For convenience, the latter molecule will hereafter be referred to as the 75 kDa TRITC-labeled dextran. Dextran solutions were prepared with phosphate-buffered saline (PBS) solution (Corning; 0.144 g/L KH_2PO_4, 9 g/L NaCl, 0.795 g/L Na_2HPO_4) to a final concentration of 100 μg/mL. Regenerated cellulose Ultracel® and polyethersulfone Biomax® UF membranes (MilliporeSigma) with nominal MWCO ratings between 10 and 100 kDa were obtained as circular discs (diameter = 25 mm). Prior to use, the membrane discs were soaked in distilled, deionized water for at least one hour to hydrate the membrane structures; a 10% ethanol/water mixture was used as a storage solution.

2.2. Stirred cell experiments

Conventional stirred cell experiments were conducted in an Amicon® 8010 stirred cell (MilliporeSigma) over a magnetic stir plate set to a nominal rotational speed of 400 rpm, a value that was determined empirically. The stirred cell was filled with 10 mL of the dextran solution and pressurized with nitrogen gas. A minimum of 1 mL of permeate was purged, or the cell was run for two minutes (whichever took longer), and then five consecutive permeate samples of approximately 150 μL (each) were collected. The average permeate flux was determined from the timed collection of these permeate samples, which were subsequently weighed using an analytical balance. Between experiments, the stirred cell was extensively flushed with PBS solution.

2.3. High-throughput stirred cell experiments

HTSC experiments were conducted by first punching smaller membrane discs from the as-received membranes using a 14-mm arch punch, and then seating them into each cell. The cells were filled with dextran solution using a long (~10 cm) needle that passed through the feed port; entrapped air bubbles were displaced by the injected fluid. A multi-rack syringe pump (Harvard Apparatus PHD Ultra) was used to fill each cell with dextran solution and then to establish the desired permeate flux for the filtration