1.1 The Rotor

The critical role of the rotor in the preparative-scale DFGF design spurred us to rigorously test the planned rotor design prior to fabricating the rest of the apparatus. This allowed the rotor's design to be optimized at minimal cost without re-designing the entire device. There were three principal requirements for the rotor. First, it needed to have a high thermal conductivity to minimize the resistance to heat transfer between the separation annulus and the cooling buffer in the rotor's lumen. Second, the rotor needed to have an effective conductivity of at least 1% of the buffer's conductivity in order to limit the loss of peak voltage within the separation annulus to less than 5% of the voltage applied on the electrode array in the rotor (1). And third, the rotor had to retain proteins in the separation annulus.

Military grade AX05 boron nitride (Saint-Gobain, Amherst, NY, USA) was selected as the first choice for a rotor material because it has a high thermal conductivity (130 W/m/K) and its 13% porosity should allow current to pass through the rotor (1). However, the ceramic's effective conductivity needed to be measured to see if it fell within an acceptable range, 1% of the buffer's conductivity or greater, in order to proceed with the design. Also, there was no data available on the pore size of the ceramic that could be used to estimate whether proteins could enter the ceramic. Therefore, a test chamber was built to work with short discs of boron nitride (2.54 cm outer diameter (OD) x 1.27 cm inner diameter (ID) x 0.635 cm high) in order to measure the effective conductivity and protein rejection of the various boron nitride disc and membrane combinations.

Testing the small boron nitride discs (data not presented) showed that boron nitride had an effective conductivity that was 1.1% of the buffer's conductivity, which fell in the acceptable range. However, bare boron nitride failed to reject cytochrome c, a 12 kD red-colored test protein, when a radial electric potential (10 V) was used to drive the protein towards the center of the disc. Filling the

pores with a highly cross-linked gel that would exclude proteins seemed like a natural solution to keeping proteins out of the rotor. Polyacrylamide gel electrophoresis showed that cytochrome c couldn't migrate in a 50% acrylamide gel (data not shown), so the pores of the boron nitride were filled with 50% acrylamide gel. Unfortunately, filling the pores with acrylamide dropped the rotor's effective conductivity to 0.0027% of the buffer, which was outside acceptable design range. Further testing with more boron nitride discs showed (data not presented) that coating the ceramic with a 0.25 mm thick polysulfone membrane (see P-P membrane in Table 2 of (2) for membrane recipe) reduced the effective conductivity to 1.0% of the buffer. The polysulfone membrane also achieved 99.7% rejection of cyctochrome c, a 12 kD protein, after 39 hours with a radial electric potential (10 V) driving the protein towards towards the center of the disc (data not shown). Thus, AX05 grade boron nitride with a 0.25 mm polysulfone membrane was selected for use in the rotor because it met the requirements for thermal and electrical conductivity and protein rejection.

Coating the rotor in polysulfone posed another problem. Applying a uniform coat of membrane to the shaped rotor was impossible with the resources available to us. Using the boron nitride rotor coated with a polysulfone membrane would require removing the shaping on the rotor that was intended to control dispersion by generating stabilizing vortices. We needed to verify that a smooth rotor with a grooved stator would still produce stabilizing vortices before accepting the smooth, membrane-coated rotor as the final design. The model presented by Ivory (3) for transport in shaped vortices was altered to solve the Navier-Stokes equations for fluid flow in an annulus with a smooth rotor and shaped stator. Results from that model (not presented) showed that the vortices still formed over a range of rpm, which meant the smooth, polysulfone-coated boron nitride rotor could be used with a grooved stator without losing the stabilizing vortices.

1.2 Cooling System

The cooling system comprised a pump, a heat exchanger, and a chiller that provided the cold buffer needed to dissipate Joule heat produced during DFGF. Previous modeling work (1) suggests that the cooling system should be designed to remove 1 kW of Joule heat, which requires 25 L/min of buffer cooled to 5 °C to flow through the rotor's lumen. A magnetically driven March AC-5C-MD pump (March Manufacturing, Glenview, IL, USA) circulated cooling buffer from a 20 L reservoir through the heat exchanger, the degassing unit, the rotor's lumen, and finally back to the reservoir. The March AC-5C-MD pump can supply 25 L/min so long as the total pressure drop does not exceed 71.7 kPA (10.4 psi). However, the VWR 1197 chiller (VWR, S. Plainfield, NJ, USA) could only provide 825 W of cooling. This limitation meant that low-conductivity buffers, less than 0.032 S/m, had to be used to avoid excessive heat generation at the planned peak field strength of 35 kV/m (1). The custom made shell and tube heat exchanger (figure 1) was designed to use 15 L/min of 40% ethylene-glycol at 0 oC on the shell side to remove 920 W from the cooling buffer flowing at 25 L/min through the tubes. Limitations on the power supply, which will be explained later, eliminated the need to actually do 825 W of cooling. Therefore, the chiller was filled with deionized water and set to 5 °C. The heat exchanger could still do 530 W of cooling in this configuration and kept the cooling buffer reservoir at 8 °C during experiments. The counter-flow buffer contained in a 250 mL glass bottle was chilled to 17 °C using a generic thermo-electric soda can cooler.

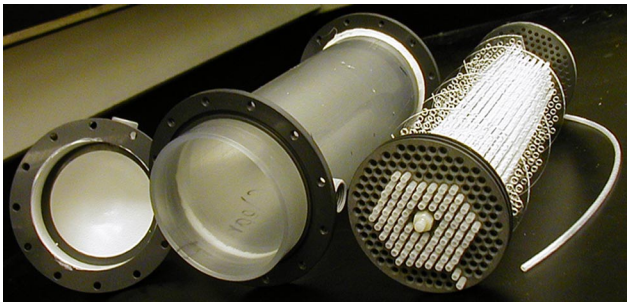

Figure 1. The custom shell and tube heat exchanger was made entirely from plastics so that it would not conduct electricity. The polycarbonate shell had a 10.16 cm ID and length of 33 cm. The tube bundle, unfinished in this photograph, had 187 low density polyethylene tubes and 6 baffles. Individual tubes had a 4 mm OD and 0.65 mm wall thickness. Tubes were sealed to the face of the tube bundle with a waterproof and chemically resistant epoxy: BONDiT-B45 (Reltek, Santa Rosa, CA, USA). A pair of o-rings at each end of the tube bundle sealed the bundle inside the shell. The end caps bolted onto the shell and sealed with o-rings.

1.3 Electrolysis Gas Removal

Electrical current creates oxygen and hydrogen gas at the anode and cathode, respectively, as it passes from the anodes into the buffer and back into the cathode. Pumping buffer over the electrodes removed the gas bubbles that would have otherwise built up on the electrodes and hindered the passage of current. A March model 1A-MD ½ pump circulated 10 L of buffer over the ground electrodes housed in the top of the stator. The cooling buffer pumped through the rotor's lumen purged the gas generated on the electrode array. However, simply entraining macroscopic gas bubbles would still allow them the chance to settle on the inner wall of the lumen and occlude the pores, reducing the conductivity of the rotor. Degassing the cooling buffer prior to flowing it over the electrodes would help dissolve the oxygen gas in the buffer and minimize the formation of macroscopic bubbles. Therefore, the cooling buffer was passed through the fiber-lumen of a hollow-fiber module, shown in figure 2, (Spectrum Laboratories, Rancho Dominguez, CA, USA) with a 71 kPa vacuum pulled on the shell side to draw the gases out of the buffer in the tubes. The hollow-fiber unit was designed to strip 60% of the dissolved oxygen and 80% of the hydrogen in one pass through the module at 25 L/min. The oxygen removal rate, 0.029 mol/min, exceeded the expected generation rate on the electrode array by 200x, based on the 870 mA drawn in simulated protein separations (1). This gave the buffer plenty of capacity to dissolve the oxygen produced on the electrode array and prevent bubbles from blocking the rotor's pores.

Figure 2. The Kros Flo Plus hollow-fiber unit contained 10 m^2 of polypropylene fibers that were 65 cm long and had a 240 micron OD with 20 micron wall thickness. Buffer flowed though the fibers while a 71 kPa (21" Hg) vacuum was maintained on the shell side.

References

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