Ultrafiltration characteristics of plasmid DNA

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Introduction

Plasmids are extra-chromosomal circular double-stranded DNA molecules that can be used to develop recombinant cell lines and to deliver therapeutic genetic material. In gene therapy applications, the *in-vivo* insertion of the genetic vector results in the production of a desired therapeutic bio-molecule. Gene therapy is of interest in the treatment of a wide range of medical conditions including cystic fibrosis, haemophilia, and muscular dystrophy. The plasmids used in clinical trials typically range in size from 5 to 20 kilo-base pairs (kbp), which corresponds to a size on the order of 100 nm.

Although small-scale laboratory techniques for the purification of plasmid DNA are well established, many of the existing methods would difficult or too expensive for large-scale commercial production of plasmid DNA. There is thus considerable interest in the development of new and improved methods for purification of plasmid DNA. This includes the possible use of membrane ultrafiltration (UF), which is a well-established unit operation for the large-scale production of recombinant therapeutic proteins.

Previous studies have identified a number of factors that affect the transport of nucleic acid (RNA, chromosomal DNA, and plasmid DNA) through microporous membranes. Hancher and Ryon [1] published the first study on nucleic acid filtration examining the behavior of a ribonucleic acid (RNA) during stirred cell ultrafiltration. RNA transmission increased with increasing membrane molecular weight cut off (MWCO), and hence the membrane pore size. In contrast, Hirasaki et al. [2] reported no significant difference in the sieving behavior of a 3 kbp plasmid through UF membranes with 15 and 35 nm pores. The reason for this discrepancy is unclear. Kahn et. al. [3] investigated the sieving behavior of plasmids during tangential flow filtration (TFF). The results showed a significant loss of plasmid into the filtrate at high transmembrane pressures. Eon-Duval et al. [4] investigated the effects of membrane pore-size and solution conductivity on the retention of a 7.7 kbp plasmid by UF membranes in a TFF process. Plasmid retention was a function of both the membrane MWCO of 300 to 500 kDa and the solution conditions, with the observed retention increasing with increasing solution ionic strength.

Although these studies have demonstrated the potential of using ultrafiltration for plasmids, there is still no quantitative understanding of the key phenomena governing the transport of plasmids through semi-permeable ultrafiltration membranes. The objective of this study was to obtain quantitative data on the effects of solution environment and filtration conditions on plasmid ultrafiltration, with these data used to develop a more fundamental understanding of the physical phenomena controlling plasmid transmission.

Experimental

Ultracel composite regenerated cellulose membranes with nominal molecular weight cutoffs of 100, 300, and 1000 kDa were obtained from Millipore Corp. (Bedford, MA). A 3000 base pair (bp) pBluescript plasmid was obtained from Invitrogen in a Tris-EDTA buffer. Agarose gel electrophoresis verified that >95% of the plasmid was in the desired supercoiled form.

Buffer solutions were prepared by dilution of a stock solution of Tris-EDTA with deionized distilled water. Sodium chloride was then added to achieve NaCl concentrations of 1, 10, 40, or 150 mM. The plasmid feed solution was prepared by dilution of the plasmid stock solution with the appropriate amount of buffer to obtain a final plasmid concentration of 250 ng/mL. Plasmid concentrations were evaluated using a PicoGreen DNA fluorescent assay with samples analyzed on a GENios FL microplate reader.

Filtration experiments were performed using 10 mL (4.1 cm^2 area) stirred ultrafiltration cells (Millipore Corp.). The membrane disc was first pre-conditioned by immersion in a 250 ng/mL plasmid solution for 24 hours at room temperature. There was no evidence of any plasmid adsorption to the Ultracel membranes. Sieving experiments were conducted over a range of permeate flux, set by adjusting the air pressurization on the feed reservoir, and at a constant stirrer speed of 730 rpm. Filtrate samples were taken after a minimum of 2 minutes or after collection of at least 1 mL of filtrate, whichever occurred first. The permeate flux was evaluated using timed collection. All feed and permeate samples were stored at 4°C for subsequent analysis. The observed sieving coefficient was calculated as the ratio of the concentration in the permeate to that in the feed:

$$S_o = C_{permeate} / C_{feed}$$

Feed and permeate samples were also analyzed by agarose gel electrophoresis to ensure that there was no degradation of the plasmid during the ultrafiltration experiment.

Results

Typical data for the sieving coefficient of the 3000 bp plasmid through a 300 kDa membrane at an ionic strength of 10 mM NaCl are shown in Figure 1. The observed sieving coefficient at low filtrate flux (below about 20 μm/s) was less than 0.01. The plasmid sieving coefficient increased rapidly at fluxes above 20 μm/s, attaining a value of more than 0.70 at a filtrate flux of 120 μm/s. The filtrate flux varied linearly with transmembrane pressure over the range of flux examined in Figure 1 (data not shown), which is consistent with the very low DNA concentration (250 ng/mL) used in this experiment. There was no evidence of any fouling during the plasmid ultrafiltration experiment, with the permeability of the membrane after the ultrafiltration always within 10% of that for the clean Ultracel membrane.

Although the increase in sieving coefficient at high flux seen in Figure 1 is consistent with the effects of concentration polarization, the data could not be described using any of the classical concentration polarization models. Instead, the sharp increase in plasmid

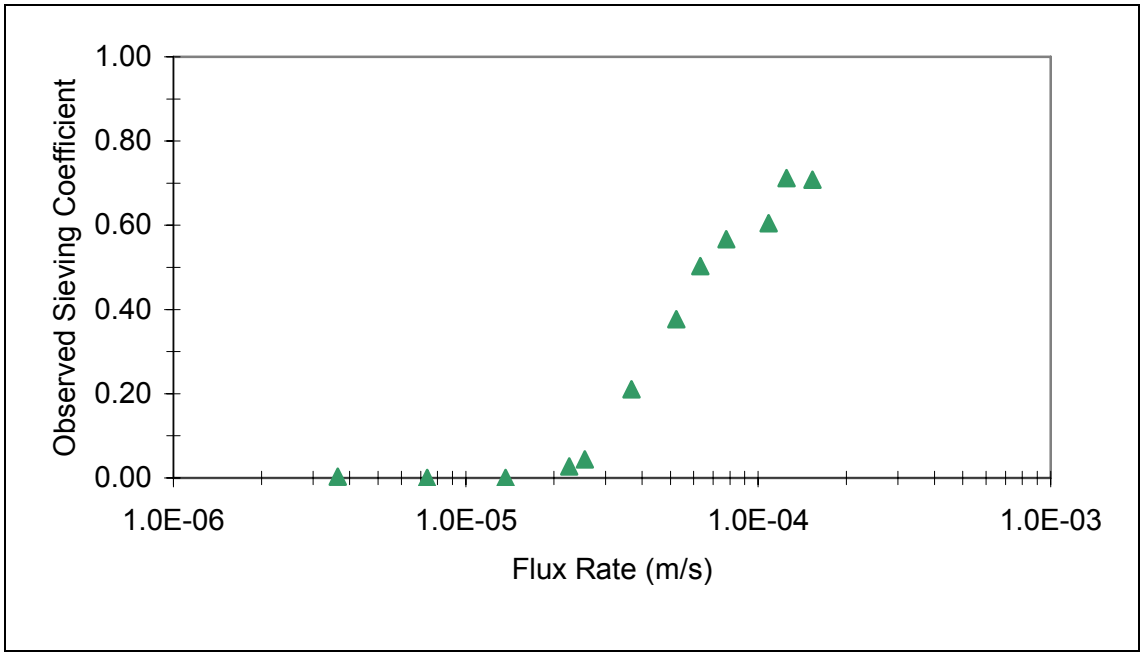

Figure 1: Effect of filtrate flux on plasmid transmission through a 300 kDa membrane in a 10 mM NaCl solution

transmission may be due to some type of flow-induced orientation or conformational change in the plasmid DNA, allowing the plasmid to pass fairly easily through the membrane at high flux.

The effect of the NaCl concentration on the plasmid sieving coefficient through the 300 kDa membrane is examined in Figure 2. Plasmid retention is nearly 100% at low filtrate flux, independent of the NaCl concentration. At each salt concentration, the sieving coefficient increases with increasing filtrate flux, with the "critical flux" at which plasmid transmission first

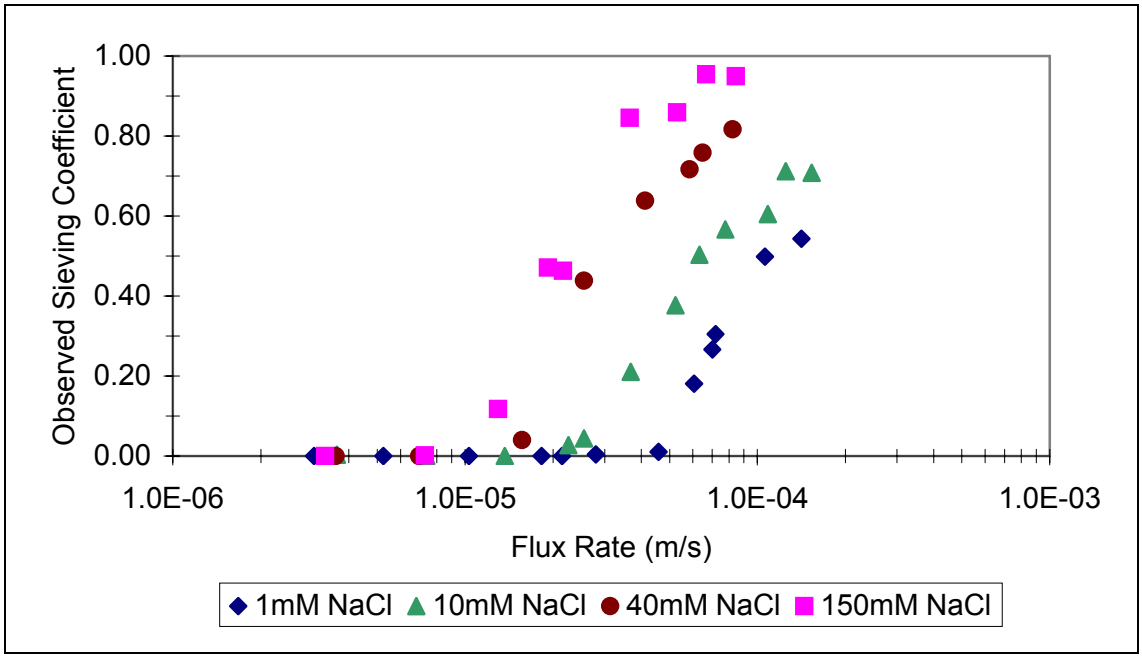

Figure 2: Effect of NaCl concentration on plasmid transmission through the 300 kDa membrane

becomes significant increasing with decreasing NaCl concentration. The net result is that the plasmid sieving coefficient at a flux of 40 μm/s increased by more than 80 fold as the NaCl concentration was increased from 1 to 150 mM.

The increase in plasmid transmission at high NaCl concentrations suggests that the effective plasmid size is reduced in the present of salt. This behavior is in good agreement with independent studies of the properties of plasmid DNA in free solution. For example, Borochov et al. [5] used light scattering to evaluate the radius of gyration of a 6.5 kbp plasmid, with the measured radius decreasing by 24% as the NaCl concentration increased from 5 to 200 mM. Lyubchenko and Shlyakhtenko [6] used AFM to examine the geometry of a 3.8 kbp plasmid in a Tris-EDTA buffer with and without NaCl. The plasmid structure was very different in the two solutions, with the structure in the presence of 160 mM NaCl characterized as being 'tightly twisted' with a smaller effective size.

Data were also obtained for the plasmid sieving coefficient using UF membranes with different nominal molecular weight cut-offs. Results for 100, 300, and 1000 kDa Ultracel membranes using a plasmid in Tris-EDTA plus 10 mM NaCl are shown in Figure 3. The plasmid sieving coefficients are significantly larger for the larger molecular weight cut-off membrane; the sieving coefficient through the 1000 kDa membrane at a filtrate flux of 50 μm/s was 100 times larger than that through the 100 kDa membrane at a comparable flux. In addition, the critical flux for any measurable plasmid transmission decreases dramatically with increasing membrane molecular weight cut-off, going from around 50 μm/s for the 100 kDa membrane to 8 μm/s for the 1000 kDa membrane.

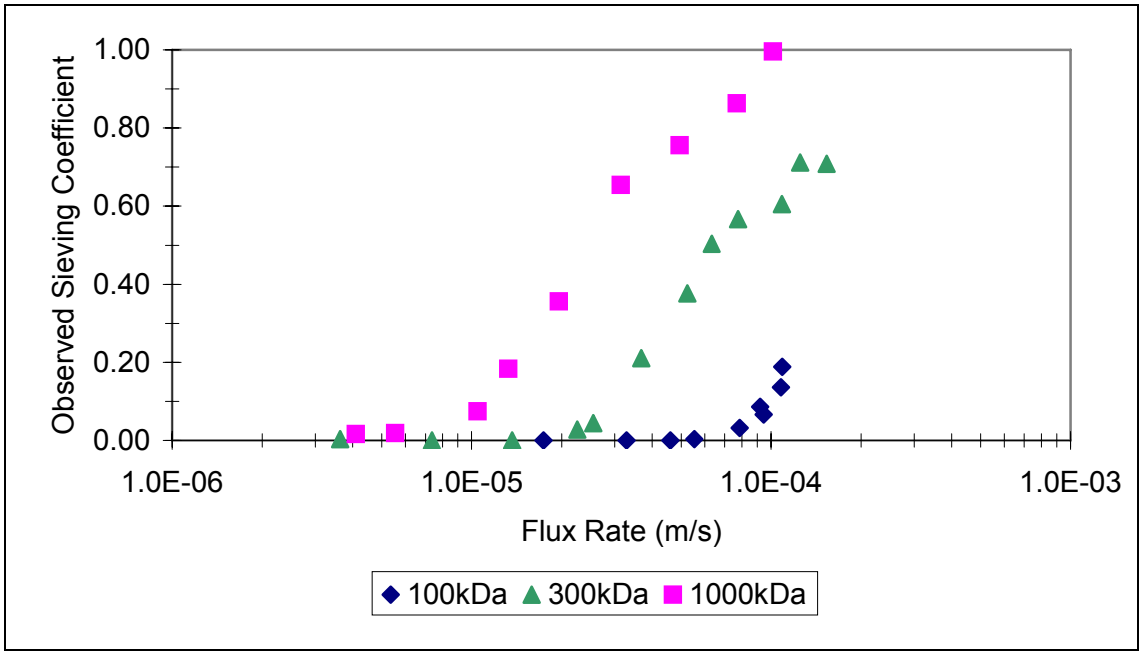

Figure 3: Plasmid transmission as a function of the filtrate flux through 3 different molecular weight cut-off membranes using a 10 mM NaCl solution

Discussion

The results obtained in this study provide some of the most quantitative data currently available for the effects of solution conditions, membrane pore size, and filtrate flux on the transmission of plasmid DNA during ultrafiltration. The plasmid was nearly 100% retained at low filtrate flux, even when using a 1000 kDa membrane. However, the transmission increased dramatically at high flux, which appears to be due to either a flow-induced orientation of conformational change in the plasmid DNA. The sieving coefficient also increases with increasing solution ionic strength, with this behavior consistent with independent studies showing a reduction in the effective plasmid size in the presence of salt. These results clearly indicate the potential of using ultrafiltration for the concentration and / or purification of plasmid DNA, with the extent of plasmid transmission controlled by adjusting the operating conditions and the choice of membrane pore size.

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