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Adsorption and desorption behavior of plasmid DNA on ion-exchange membranes Effect of salt valence and compaction agents

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Abstract

In this study, we report the effect of salt type and compaction agents on adsorption and desorption behavior of plasmid DNA on strong anion-exchange membranes. Both divalent cations and compaction agents are known to reduce the effective charge density of plasmid DNA in solution, and compaction agents decrease the radius of gyration of plasmids. Differences in the batch uptake adsorption of a 6.1 kilo base pair plasmid in solution with sodium and magnesium salts were observed at low ionic strengths. Recoveries at high salt conditions, however, were independent of the cation, and measured only 63–76%. Similarly, no improvement in recoveries were observed when using sulfate rather than chloride anions as displacers. The compaction agents, spermine and spermidine, showed no strong effect on the uptake adsorption, capacity, or recovery of three different-sized plasmids on membrane sheets. It is recommended that further efforts to improve plasmid recoveries from anion-exchange membranes focus on properties of the adsorbent surface.

Keywords: Adsorption; Membranes; Salt effects; DNA

1. Introduction

Adsorptive membranes have been shown to offer advantages over traditional porous bead resins for purification of large biomolecules such as plasmid DNA [1,2]. The large convective pores found in membranes result in small diffusion distances as well as a large surface area accessible to plasmids, giving a large capacity. These characteristics are in strong contrast with conventional bead resins, where capacities for plasmids are limited by exclusion from pores or very long diffusion times into the pores [3–5]. However, recovery of plasmid DNA from strong anion-exchange membranes is low, and needs to be improved to fully realize advantages of the adsorbent [6]. The large polyanionic plasmids can form very strong multi-point attachments with the ion-exchange surface, and limit recoveries to the order of 50–80%, depending on the operating conditions. As electrostatic forces dominate in ion-exchange chromatography, we would like to understand and manipulate the variables that control the magnitude of these forces in an effort to improve the reversibility of plasmid binding.

Insight into the electrostatic interaction between oppositely charged bodies in contact with an electrolyte solution has been obtained for simple geometries from solutions of the Poisson–Boltzman equation (see [7–9]). For two oppositely charged slabs, the change in free energy needed to move the surfaces from an infinite distance to a distance L from each other is found to be [8]:

$$\Delta G(L) = \frac{A_\mathrm{p}}{\kappa\varepsilon_0\varepsilon_\mathrm{r}} \cdot \left(\frac{(\sigma_\mathrm{p}^2 + \sigma_\mathrm{s}^2)\mathrm{e}^{-\kappa L} + 2\sigma_\mathrm{p}\sigma_\mathrm{s}}{\mathrm{e}^{\kappa L} - \mathrm{e}^{-\kappa L}} \right) \quad (1)$$

where A_p is the area of interaction, σ_p^2 and σ_s^2 are the surface charge density of the respective slabs, ε_0 the permittivity of a vacuum, ε_r the dielectric constant of the solvent, and $1/\kappa$ the Debye length or thickness of the ionic atmosphere. The minimum free energy between the two surfaces is only dependent on the surface carrying the lower charge density, and is given by [8]:

$$\Delta G_\mathrm{min} = -\frac{A_\mathrm{p}\sigma_\mathrm{min}^2}{\kappa\varepsilon_0\varepsilon_\mathrm{r}} \quad (2)$$

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The free energy dependence on the solution ionic strength I comes through the parameter κ, which is defined as:

$$\kappa = \frac{F\sqrt{\sum_i c_i z_i^2}}{\sqrt{\varepsilon_0 \varepsilon_r RT}} = \frac{F\sqrt{2I}}{\sqrt{\varepsilon_0 \varepsilon_r RT}} \quad (3)$$

where F is Faraday's constant, R the universal gas constant, T temperature, c_i the molar concentration of each ionic species, and z_i the valence of the respective ion. It follows that at a given temperature, the minimum free energy between two charged surfaces is proportional to the area of interaction, the square of the least densely charged surface, and the inverse square root of the ionic strength:

$$\Delta G_{min} \propto \frac{A_p \sigma_{min}^2}{\sqrt{I}} \quad (4)$$

For modeling the retention of ions in chromatography using the above description of two charged slabs in an electrolyte solution, a relation between the free energy and a retention factor, or the distribution of solute ions between two phases, is necessary. The retention factor k, defined as the equilibrium ratio of solute mass in the adsorbed and liquid phases, has been approximated as [10]:

$$\ln k = -\frac{\Delta G_{min}}{RT} + \ln\left(\frac{A_s d}{V_0}\right) \quad (5)$$

where A_s is the surface area of stationary phase, V_0 the dead volume of the column, and d the characteristic width of the adsorption layer. The second term in Eq. (5) is invariant to changes in ionic strength over typical operating conditions, giving a linear relationship between the logarithmic retention factor and minimum free energy. While the above expressions represent idealizations not always found in quantitative agreement with reality, they have proven useful guides. We thus aim to control the binding strength and behavior through manipulating the charge density and size of the solute.

The size and effective charge of DNA molecules can be altered by the presence of multivalent cations or compaction agents in solution [11]. Changes in size have been characterized by methods including light scattering [12], atomic force microscopy [13], and electron microscopy [14]. Fractional charge neutralization has been verified by gel electrophoresis [15]. According to Manning's counterion condensation theory [16], counter-ions will condense on a polyelectrolyte to decrease its linear charge density to a limiting value. For the simple case of a solution containing B-DNA and only one species of counter-ion with charge Z, the linear charge density will be reduced by a factor r of [16]:

$$r = 1 - \frac{1}{Z\zeta} \quad (6)$$

where ζ is given by

$$\zeta = \frac{q_p^2}{\varepsilon kTb} \quad (7)$$

Here, q_p is the protonic charge and b is 1.7 Å, the spacing between charges of B-DNA, so ζ equals 4.1 in an aqueous solution at 25 °C. Following Eq. (6), monovalent cations reduce B-DNA charge by 76% and divalent cations by 88%. For DNA in the presence of an excess of two cations, the charge density is not only a function of the valence but is dependent on the concentration of both counterions [12,16].

Furthermore, experimental evidence has shown that DNA condenses into a compact structure when approximately 90% of its charge is neutralized by counterions [11]. These counterions or condensing agents decrease repulsions between DNA phosphates and typically require a charge of +3 or greater to condense DNA in aqueous solutions. The most common compaction agents used include spermidine (3+), spermine (4+), and hexamine cobalt (3+). Anion-exchange chromatography of DNA in solution with the above compaction agents was recently investigated [17], showing that the surface capacity of anion-exchange beads could be increased by as much as 40% under suitable ionic strength conditions. Compaction of plasmid DNA to a level allowing either increased access to the resin pores or closer packing on the outer particle surface explained the observed increase in capacity.

In this study, we tested the hypothesis that the recovery of plasmid DNA from strong anion-exchange membranes would be improved if the effective charge density of the plasmid and the area of interaction with the surface were reduced. Both multivalent cations and compaction agents are known to contribute to reduce the charge density and radius of gyration of plasmid DNA, and loading and eluting under such conditions may alter observed chromatographic behavior. Although the capacity of membranes for plasmids are not limited by exclusion from small pores, changes in how the plasmids are packed on the surface may have an effect on the capacity.

2. Experimental

2.1. Materials

All chemicals used were from Sigma (St. Louis, MO, USA) and buffers were prepared with 18 MΩ water. The compaction agents, spermine (97% pure) and spermidine (99% pure), were purchased from Acros Organics (Somerville, NJ, USA). Sheets of Mustang-Q anion-exchange membranes supplied by the manufacturer (Pall, Ann Arbor, MI, USA) were cut and used for batch adsorption and desorption. The Mustang-Q membranes are polyethersulfone (PES) membranes chemically modified to produce a quaternary amine surface. The nominal pore size of the PES membranes is 0.8 μm and the thickness of each membrane layer is 70–90 μm. Frozen *Escherichia coli* cell paste containing a 6.1 kilo base pair (kb) plasmid DNA (pCI-luc-kan plasmid, 6103 bp) was obtained from the Waisman

Clinical BioManufacturing Facility (Madison, WI, USA) and purified using the procedure previously described [1]. The 4.5 kb (pCAT) and 14.5 kb (pMS14) plasmids were obtained in a purified state from Pall. A Cary UV–Vis spectrophotometer (Palo Alto, CA, USA) was used for concentration measurements in batch experiments at 260 nm.

2.2. Procedures

Static batch adsorption and desorption: Sheets of Mustang-Q anion-exchange membranes were cut into pieces with a cross-sectional area of 0.5 cm^2. The membranes were cleaned in a 1 M NaOH solution, regenerated in 1 M NaCl, and equilibrated in a solution (50 mM Tris–1 mM EDTA buffer, pH 8.0) with the desired salt type (sodium chloride, magnesium chloride, sodium sulfate, and magnesium sulfate) and ionic strength (I = 0.1, 0.5, and 0.7 M) for the batch uptake experiments. Plasmid solutions with the respective salt types and ionic strengths were prepared at concentrations of 20, 40, 60, 80, and 120 μg/ml all in 1.5 ml microcentrifuge tubes. The respective membrane sheets were subsequently added. The plasmid solution and membranes were equilibrated on a shaker table at 280 rpm and ambient temperature overnight, after which no more adsorption was observed. The equilibrium liquid-phase plasmid concentration was measured by absorbance at 260 nm (extinction coefficient = 0.02 $cm^2/\mu g$), and the mass of plasmid bound to the membrane was determined by an overall mass balance. Solid-phase plasmid concentrations are reported as microgram per cross-sectional membrane sheet area. The batch experiments were done in triplicate for each plasmid loading with each salt type and ionic strength. Uptake curves were fitted to the Langmuir isotherm, written in the form:

$$q = \frac{q_{\max} c}{K_D + c} \tag{8}$$

It should be noted that assumptions of the model do not hold for plasmid adsorption, namely the strong observed hysteresis. However, it is used for as an empirical fit for ease of interpretation of the capacity q_{max} and concentration at half saturation K_D. Elution of the plasmid from the membrane sheets occurred in the respective salt solution with an ionic strength of 1.5 until the plasmid concentration reached a steady state. The mass of plasmid eluted was measured by absorbance at 260 nm and recoveries were calculated.

A similar procedure was followed for batch uptake and desorption experiments in the presence of compaction agents. In each case, a concentration of 2.5 mM of the respective compaction agent was added to the uptake (0.5 M NaCl) and desorption (1.5 M NaCl) solutions. All runs were done in duplicate for the three different-sized plasmids.

3. Results and discussion

3.1. Effect of salt valences

The effect of ionic strength and salt type on batch uptake adsorption of a 6.1 kb plasmid was determined using both monovalent and divalent salts. The concentration of plasmid adsorbed to the membrane q is plotted against the final liquid-phase concentration c in Fig. 1. Batch uptake occurred at ionic strengths of 0.1, 0.5, and 0.7 M for each of the four salts—NaCl, $MgCl_2$, Na_2SO_4, and $MgSO_4$. A non-linear fit of each data set to the Langmuir isotherm yielded the parameters q_{max} and K_D. The effect of salt type and ionic strength on binding strength is presented in Fig. 2, where q_{max}/K_D (proportional to k in Eq. (5)) are plotted on a log scale against the inverse square root of the ionic strength for each salt.

3.1.1. Effect of cation

As described by Manning's counterion condensation theory, DNA will carry a smaller effective charge when in presence of divalent cations rather than monovalent cations. According to Eq. (2), it would follow that the minimum free energy and thus, chromatographic behavior of DNA would change when carrying a lower charge density. As seen in Figs. 1 and 2, changes in adsorption uptake behavior using the divalent magnesium salts were only observed at the lowest ionic strength studied. Here, plasmid uptake in both magnesium salts (squares in Fig. 2) at I = 0.1 M showed a lower binding strength compared to the uptake in sodium salts (triangles in Fig. 2) at the same ionic strength, an observation consistent with a reduced charge density. Behavior at intermediate ionic strengths, however, did not show any trends related to the valence of the cation, and in all cases, the observed capacity q_{max} was unchanged.

The reversibility of binding is displayed in Fig. 3, where the recovery is plotted for each salt type. Here, data are for plasmids loaded at ionic strengths of 0.1 and 0.5, and eluted at an ionic strength of 1.5 in the respective salt solutions. It should be noted that increases in recovery were not observed at ionic strengths greater than 1.5 for any salt. No statistical difference in recovery was observed when comparing the sodium salts with the respective magnesium salts. While the charge density of DNA in an aqueous solution of divalent cations is less than that with monovalent cations, it may not hold true when adsorbed to the ion-exchange surface. The local environment of the positively charged quaternary amine surface is less favorable for the cations responsible for shielding the DNA charge, and this could explain the insensitivity of the plasmid reversibility to the cation in solution.

3.1.2. Effect of anion

The effect of the displacing anion on batch uptake is also presented in Fig. 2, where sulfate salts (open symbols) are contrasted with chloride salts (closed symbols). The most notable difference is at the highest ionic strength, I = 0.7, where significant plasmid binding still occurred in both

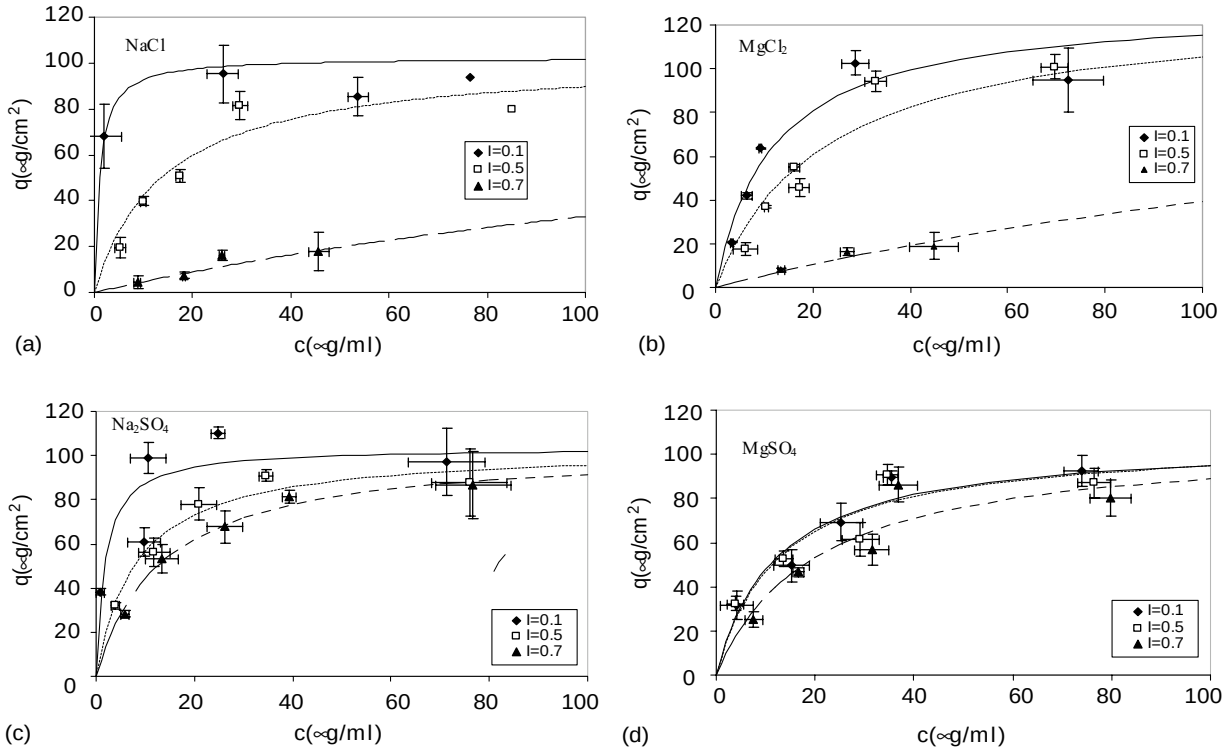


Fig. 1. Batch adsorption uptake of a 6.1 kb plasmid DNA on strong anion-exchange membranes at different ionic strengths in (a) NaCl, (b) $MgCl_2$, (c) Na_2SO_4, and (d) $MgSO_4$ solutions.

of the sulfate salt solutions and minimal binding occurred in both of the chloride salt solutions. If the anion has no effect on the size or charge density of the plasmid, Eq. (4) predicts that adsorption and desorption behavior should be only a function of the ionic strength and independent of the salt type. The type of displacer salt, however, is known to affect the adsorption and desorption behavior in the anion-exchange chromatography of proteins and peptides [18–21]. Alternative models suggest that retention is not controlled by solution ionic strength, but rather the concentration and valence of the displacing ion [22] or the displacing ionic strength [20]. Empirical eluotropic series show sulfate ions as having greater binding affinity and elution strength. Despite these observations, the recovery of plasmid from the anion-exchange membranes was independent of the anion used in loading and elution of the plasmid.

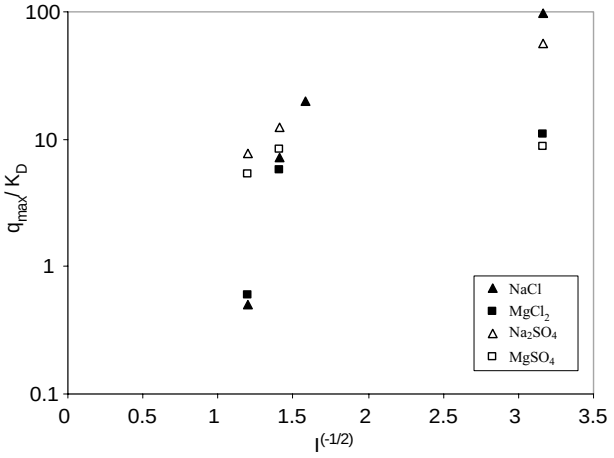


Fig. 2. The effect of salt type on initial binding strength of 6.1 kb plasmid DNA to strong anion-exchange membranes.

3.2. Effect of compaction agents

The effect of the compaction agents spermine and spermidine on the uptake of 4.5, 6.1, and 14.5 kb plasmids was studied at an ionic strength of 0.5 M in sodium chloride,

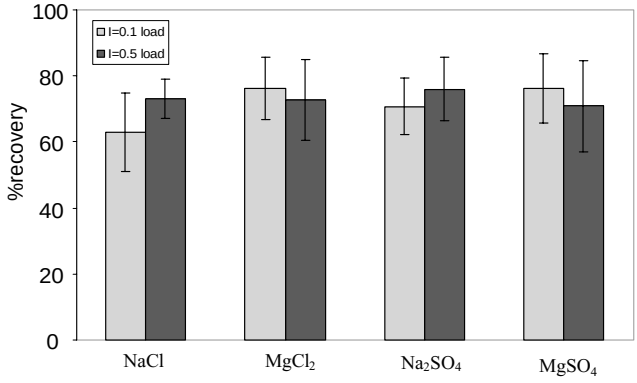
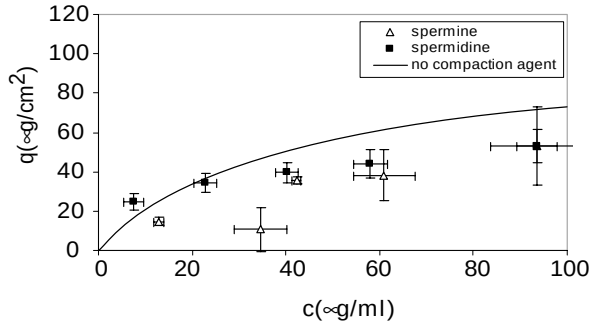
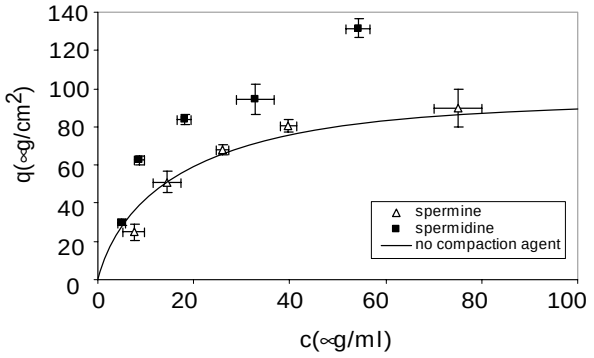


Fig. 3. The effect of salt type on recovery of 6.1 kb plasmid DNA from strong anion-exchange membranes. An ionic strength equal to 1.5 of each respective salt solution was used to elute the plasmid.

(a) 4.5 kb plasmid



(b) 6.1 kb plasmid



(c) 14.5 kb plasmid

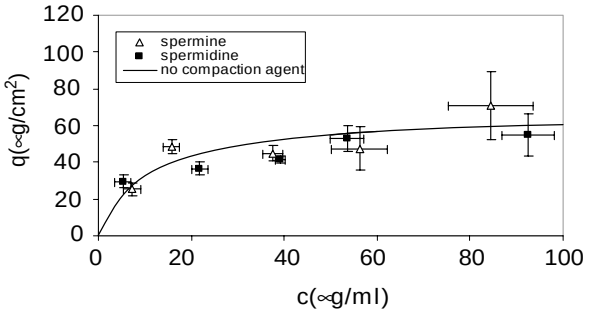


Fig. 4. Batch uptake adsorption of (a) 4.5 kb, (b) 6.1 kb, and (c) 14.5 kb plasmid DNA on quaternary ion-exchange membrane sheets in presence of 2.5 mM spermine (open triangles) and 2.5 mM spermidine (squares), all in solution with 0.5 M NaCl. Lines represent previous fits to uptake without compaction agents.

and the data are plotted in Fig. 4 along with a fit to previous uptake data at the same ionic strength without compaction agents. The adsorption uptake of the 4.5 kb plasmid with spermine and spermidine was only slightly lower than that observed without the compaction agent. Salt conditions used were not favorable for strong adsorption and in light of typical scatter observed in the data, differences were not significant. Similarly, the largest plasmid (14.5 kb) did not show any difference in adsorption behavior in the presence of either compaction agent. The capacity of the large plasmid remained nearly 40% lower than that observed for the smaller plasmids, so any effect

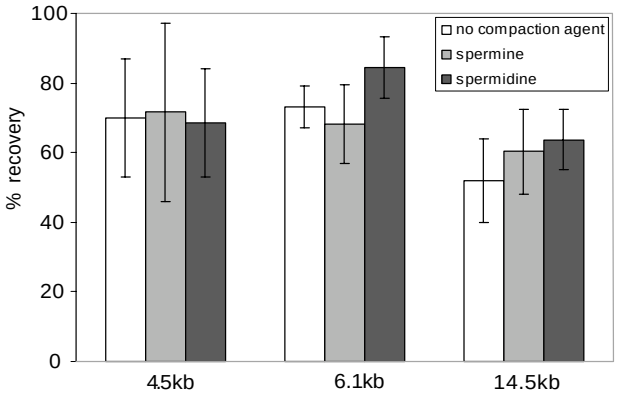


Fig. 5. The effect of compaction agents on the recovery of three different-sized plasmids from quaternary ion-exchange membrane sheets.

the compaction agents had on the size and shape of the large plasmid was not significant enough to increase accessible surface area. The uptake of the 6.1 kb plasmid in spermidine marked the only notable change in behavior, where an increase in capacity by over 20% was observed. Further investigation is needed to understand why this behavior is deviates from that observed for both the smaller and larger plasmids as well as the 6.1 kb plasmid in the spermine solution.

The average recovery of plasmid that was loaded and eluted with and without compaction agents is presented in Fig. 5. Neither spermine nor spermidine had a significant effect on the amount of plasmid that was recovered from the ion-exchange membranes. Consistent with previous findings, the average recovery of the largest plasmid was always smaller than the two smaller plasmids.

4. Conclusions

In this study, the effect of different salts and compaction agents on the uptake and reversibility of plasmid DNA from strong anion-exchange membranes was investigated. While the plasmid uptake behavior changed in the presence of divalent cations, the reversibility showed no significant difference from that observed in the presence of monovalent cations. Similarly, the valance of the displacing anion had no effect on the recovery of plasmids from anion-exchange membranes. The effect of two compaction agents, spermine and spermidine, on the uptake and reversibility of three different-sized plasmids was investigated, and no large effects on either behavior were observed. The recovery of plasmids from strong anion-exchange membranes was not improved by using liquid-phase conditions that reduce the charge density and size of plasmids. Future studies to improve the recovery of plasmids from anion-exchange surfaces should look to adsorbents with different surface chemistries or reduced charge densities.

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