Adsorption behavior of plasmid DNA onto perfusion chromatographic matrix

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Abstract Anion exchange chromatography is the most popular chromatographic method for plasmid separation. POROS R1 50 is a perfusion chromatographic support which is a reversed phase matrix and is an alternative to conventional ones due to its mass transfer properties. The adsorption and elution of the pIDKE2 plasmid onto reversed phase POROS R1 50 was studied. Langmuir isotherm model was adjusted in order to get the maximum adsorption capacity and the dissociation constant for POROS R1 50-plasmid DNA pDNA system. Breakthrough curves were obtained for volumetric flows between 0.69 – 3.33 mL/min given dynamic capacity up to 2.3 times higher than those reported for ionic exchange matrix used during the purification process of plasmids with similar size to that of pIDKE2. The efficiency was less than 45% for the flow conditions and initial concentration studied which means that the support will not be operated under saturation circumstances.

Key words plasmid purification perfusion chromatography adsorption isotherm

Advances in plasmid DNA gene therapy and vaccines demand efficient production methods to obtain plasmid DNA pDNA with high purity. One of the major problems in conventional chromatography for plasmids is the lack of capacity of the adsorbent originally designed for protein purification. Plasmids are very large molecules and their binding is likely to occur only at the surface of the adsorbents 1,2. Plasmid DNA includes open circular plDNAoc plasmid DNA linear plDNAl and plasmid DNA supercoiled plDNAsc which are the target products recommended by Food and Drug Administration FDA as plasmid DNA vaccines. POROS perfusion chromatography columns use a perfusion mass transfer technology developed by PerSeptive Biosystem Inc. POROS perfusion chromatography columns perform protein separations 10 to 100 times faster than conventional chromatographic media without sacrificing resolution or capacity 3. This fact is possible because perfusion media are composed by through-pores 0.6 – 0.8 µm that facilitate convective intra-particle transport and diffusive pores 0.05 – 0.15 µm. This combination enables a better access of macromolecules to the inner of the particles 4.

Molecules movements from a liquid phase to an active site located inside an adsorbent are controlled by different physico-chemical mechanisms as external diffusion from the liquid phase to the external surface of the particles diffusion inside pores and adsorption-desorption on the active sites. For this reason parameters that characterize the adsorption velocity and the maximum quantity of adsorbate accumulated on the adsorbent are very important.

The adsorption-desorption equilibrium of a plasmid anion exchange process was studied by Ferreira and Montesinos-Cisneros et al. 2,5. Langmuir isotherm has been successfully used for modeling the adsorption of proteins onto ion exchangers. Langmuir isotherm model supposes monolayer adsorption homogeneous linking sites and affinity no interaction between adjacent mol-

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ecules and interaction between one adsorbate and one active site. The equation is stated as

\[ q = q_{\text{max}} \times \frac{c}{K_d + c} \]

where \( c \) is the concentration of plasmid in solution adjacent to the surface that is in equilibrium with \( q \) the solid-phase plasmid concentration \( K_d \) is the dissociation constant and \( q_{\text{max}} \) is the maximum adsorption capacity of the adsorbent.

In this paper the adsorption of pIDKE2 plasmid using perfusion chromatographic columns was studied and the adsorption isotherm parameters the breakthrough curves and the elution conditions for a pIDKE2 plasmid-POROS R1 50 system were obtained.

1 Materials and methods

Plasmid used in this study was 5.5 kbp pIDKE2 obtained by Limonta et al. method. Reagents were of analytical grade or higher from Merck Germany and Caledon Canada with 98% of purity. Membranes and matrix were from Sartorius Germany General Electric USA and Applied Biosystems USA.

Adsorption isotherms for POROS R1 50-pIDKE2 plasmid system were obtained by following Vicente Arias et al. and Dorat procedures. Experiments were done in triplicate at 20 °C.

Experiments to determine dynamic capacity were performed in a 0.5 cm diameter and 5 cm height column equivalent to 1 mL of POROS R1 50 gel. Samples were applied until saturation conditions were reached in the column. Three volumetric flows of 0.69, 1.67 and 3.33 mL/min were used by following the supplier’s suggestions. The equilibrium buffer composition was ethylene-diaminetetraacetic acid EDTA 3.24 g/L tris hydroxymethylaminomethane Tris 3.24 g/L ammonium sulfate 192.8 g/L pH 7. Initial concentration of the samples was 0.166 ± 0.05 mg/mL. Experiments were done in triplicate and were carried out at 20 °C.

The equilibrium liquid-phase plasmid concentration was measured by absorbance at 260 nm and the mass of plasmid bound to the matrix was determined by an overall mass balance. Statgraphics Centurion XV software was used to obtain the regression models. In order to select the elution solvent of pIDKE2 from POROS R1 50 an experimental design type individual categorical factor in which the dependent variable was the mass of pDNA eluted was prepared with 3 solvents as the independent variable ethanol methanol and acetonitrile 40% v/v in Tris-EDTA buffer TE 1X EDTA sodium Titrplex III 1.86 g/L Tris 1.5 g/L pH 7. The experiments were done in duplicate using 0.5 mL of gel charged with pIDKE2. Elution conditions were adjusted in a column of 1.6 cm diameter and 1 cm height equivalent to 2 mL of gel a volumetric flow of 2 mL/min and a linear velocity of 60 cm/h. A step gradient was applied between water for injection WFI and the elution solution TE 1X-40% acetonitrile v/v buffer. Regeneration process was carried out with isopropanol.

2 Results and discussion

2.1 Adsorption isotherm for POROS R1 50-pIDKE2 plasmid system

Adsorption isotherms were obtained to obtain the maximum adsorption capacity and the dissociation constant. Adsorption isotherms for POROS R1 50-pIDKE2 plasmid system are shown in Fig. 1. Langmuir model was selected to correlate solid-liquid phase concentrations because several reports suggested to use it to describe the interac-

![Fig. 1 Adsorption isotherm for POROS R1 50-pIDKE2 plasmid system](image-url)
tion of matrix-pDNA $2\times 5 \times 10$ slab. Hypothesis test was done between experiments. Statistically significant difference doesn’t exist confidence level of 95%.

Several authors transformed Langmuir model into a straight line to find the parameters from intercept and slope while others applied non-linear regression. The main problem with the linear methods is the distortion of the experimental error caused by the transformation. Linear regression considers that point’s dispersion around the line follows a Gaussian distribution and equal standard deviation for each independent variable. Those considerations are rarely true when data are transformed. Also changes in relation between dependent variable and independent variable are presented depending on the specific transformation. Avoiding these errors a non-linear regression model was adjusted with Statgraphics Centurion XV software.

The results showed that the regression coefficients were higher than 0.90 with random distribution of residues and relative error less than 15%. Analyzing the results it can be concluded that adsorption of pIDKE2 plasmid followed Langmuir model 95% confidence for the concentration range studied. Corresponding values for $q_{\text{max}}$ and $K_a$ are 5.00 g/L standard error 0.23 and 0.07 g/L standard error 0.01. The equation is now stated as

$$ q = 5.00 \times c + 0.07 + c $$

POROS R1 50 has been used for pDNA purification. Until now no report has been made to model the uptake behavior of pDNA on reverse phase matrix. Because of that the comparison was done with conventional ionic exchange matrix with similar particle size. The maximum capacity obtained in this work 5.00 g/L is higher than values reported by Prazeres et al. for Langmuir isotherm and different ionic exchange matrices. Results for Q-Sepharose High Performance Fast Flow Big Beads and Streamline SL with the corresponding pore sizes of 34 $90 \mu m$ 200 and 200 $\mu m$ for adsorption of a 4.8 kbp plasmid were between 2.5 and 3.0 g/L 11 L

Tesinos-Cisneros et al. 5 had obtained a maximum capacity of 2.46 g/L for a Q-Sepharose-4 kbp plasmid. Tarmann and Jungbauer 10 had reported a Langmuir model adjustment for 4.9 kbp plasmid-Q Sepharose HP 90 $\mu m$ and 4.9 kbp plasmid-Source 30Q 30 $\mu m$ with maximum capacities of 1.94 and 1.34 g/L when no NaCl was used. Values increased to 2.56 and 2.42 g/L using 0.4 mol/L NaCl. Meanwhile POROS R1 50 with a pore size of 50 $\mu m$ showed higher superficial area due to a wide net of tiny interconnected pores 0.05 0.15 $\mu m$ that contributed to a higher capacity.

Low values for plasmids comparing with the proteins corresponded to the suggestion that plasmids should be excluded from the porous particles adsorbed by the external surface only. In addition steric hindrance of the adsorbed plasmids exists close to the union site. For that reasons capacities for plasmid are lower than those obtained with proteins 30 111 g/L 12.

### 2.2 Dynamic adsorption capacity for POROS R1 50

Dynamic adsorption capacity from breakthrough curves was evaluated at 3 volumetric flows of 0.69 mL/min 1.67 mL/min and 3.33 mL/min. Initial concentration of the samples was 0.166 ± 0.05 $g/L$. Fig. 2 shows the typical breakthrough curves. Experiments have shown that dynamic capacities were between 1.24 and 2.32 g/L no significant differences were found with confidence level of 95%. Results were similar to those described by Afeyan et al. 3. Values were higher than those obtained with conventional exchange matrices used for plasmid purification dynamic capacity 1 g/L of resin using flows between 0.5 and 1 mL/min 13.

The efficiencies determined as the relation between dynamic capacity and the maximum capacity were in the range 27% 45%. No significant differences were found with confidence level of 95% $P$ value 0.596 4 for $F$ ratio 0.62. It means that the column cannot be operated under saturation conditions for the flow conditions stud-
showed a typical chromatogram for gradient elution using WFI and the buffer while Fig. 5 shows gel electrophoresis analysis of the eluted fraction on agarose 0.8%.

![Chromatogram](image1)

**Fig. 2** Breakthrough curves for pIDKE2 plasmid-POROS RI 50 system with a buffer equilibrium solution containing 264.8 g/L NH₄NO₃SO₄ at volumetric flows of 3.33 mL/min, 1.67 mL/min, and 0.69 mL/min.

![Chromatogram](image2)

**Fig. 3** Mean values for mass of plasmid DNA using different solvents.

![Chromatogram](image3)

**Fig. 4** Typical chromatogram for gradient elution.

Column diameter: 5 cm; gel volume: 69 mL; linear velocity: 60 cm/h; sample volume: 1100–1400 mL; temperature: 30 °C; wavelength: 254 nm; washing water for injection elution: TE 1X-40% acetonitrile; regeneration: 90% isopropanol.

![Chromatogram](image4)

**Fig. 5** Agarose 0.8% gel electrophoresis for gradient elution samples from a POROS RI 50 matrix in a packed column.

Lane 1: molecular mass standard; 2: initial sample; 3: washing with water for injection; 4–6: plasmid elution's at different acetonitrile concentrations 0–40% depending on applied gradient. Eluted fractions are plasmid DNA open circular pDNAoc, plasmid DNA linear pDNAli, and plasmid DNA supercoiled pDNAsc.

Elution of 33% of the target product pIDKE2 was observed during the washing with WFI.
according to the absorbance data. However a
great amount of plasmid pADNsc was not
observed in agarose 0.8% electrophoresis.
For the elution\% pIDKE2 recovery between 50% – 60% of
purity was observed with 18% – 25% of buffer
TE 1X-40% acetonitrile. As the buffer’s acetonitrile
concentration was 40\% the interval 18% –
25% corresponded to an acetonitrile concentra-
tion between 7.2% – 10%. For these fractions\%
67\% pIDKE2 was recovered. Unspecific adsorp-
tions of plasmid to other biomolecules\% as pro-
teins\% should exist.
The dynamic capacity at 2 mL/min was between 2
– 3 mg of the initial material/mL of gel\% below
the maximum capacity according to the adsorp-
tion isotherm for the pure pDNA.
These results can be explained considering that
interaction between the matrix and the pDNA
depends on the structure. Addition of ammonium
sulfate salt at high concentration decreases the
electrostatic repulsion among the phosphates
groups of the skeleton allowing the interaction of
those with the matrix\% whose composition is entangled in the
cross-linked polystyrene-divinyl-
benzene. Ammonium sulfate also promotes the
adsorption of hydrophilic species and it contrib-
utes to the differential retention of all the pDNA
species\% like pDNAo and pDNAsc\% due to the
effect that salt causes on the porous topology of
the matrix. The fraction obtained during the
washing step contains a majority of the pDNAo
fraction\% Fig. 5\% lane 3\% which absorbs at 260 nm
as pDNA. This fact can be explained because of the
hyper chromatic effect caused by the changes from
double bases to single filaments. As
pDNAo can be considered as a contaminant of the
target product\% this eluted fraction is not
good. Once the saline concentration is reduced
because of the washing step\% desorption of
pDNAsc is favored\% obtaining the highest recovery
when elution with buffer TE 1X-40\% acetonitrile is
done. \% Fig. 5\% lane 4 – 6\%. The amount of
pDNA obtained is similar to the values reached by
PerSeptives Biosystems Inc. \% 14\% using POROS
50 HQ.

3 Conclusions
The adsorption isotherm was obtained for a
reverse phase-plasmid system POROS R1 50-pi-
DEK2 and a Langmuir isotherm model was adjusted
as $q = 5.00 \times c$\% 0.07 + $c$. The maximum
capacity for POROS R1 50 is 1.7 – 2 times higher than those reported for ionic exchange matrix used
during the purification process of plasmids with
similar size to that of pIDKE2. The dynamic capacity of POROS R1 50 lies between 1.24 and 2.32 mg
pIDKE2/mL matrix. No significant differences
exist for 0.69\% 1.67\% and 3.33 mL/min. This value is
1.2 – 2.3 times higher than those reported for
ionic exchange matrix used during the purification
process of plasmids with similar size to that of
pIDKE2. The efficiency determined as the relation
between dynamic capacity and the maximum
capacity was less than 45% for the flow conditions
studied and initial concentration\% 0.166 ± 0.05
\% g/L. The elution conditions were established com-
bining a wash with WFI and the gradient elution
with a buffer containing 40% acetonitrile.

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