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PURIFICATION OF pDNA USING AE PERFUSIVE CHROMATOGRAPHY

Atenas Posada, UNISON, Rosales y Boulevard Luis Encinas, Hermosillo Sonora, CP 83000. Rosa María Montesinos, Depto. de Matemáticas UNISON, (rmontesinos@gauss.mat.uson.mx). Patricia Guerrero, Depto. de Ingeniería Química y Metalurgia, UNISON. Jaime Ortega (CINVESTAV-IPN Zacatenco). Armando Tejeda, DICTUS-UNISON.

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Introduction. Supercoiled plasmid DNA (scpDNA) is beginning to be commercially used as a vector in vaccines [1]. Therefore, highly productive and economic processes are required. Regulatory agencies demand a highly purified scpDNA for vaccine use, which should be accomplished with chromatography operations [2].
The purpose of this study was to purify scpDNA in a single step using frontal AE-Chromatography on columns packed with perfusive particles [3].

Methods. The plasmid pVAX1-NH36 (4000 bp) hosted in *Escherichia coli* DH5α was used. The insert NH36 codes for a protein which may confer immunity against leishmaniasis. Plasmid purification started with a clarified lysate treated with $CaCl_2$ and processed by tangential flow filtration (TFF). Then, the plasmid was purified by frontal ion-exchange chromatography using the adsorbent POROS 50 HQ in Tris 20 mM/HCl, 0.5 M NaCl, buffer at pH 8. The scpDNA elution was carried out using Tris 20 mM/HCl pH 8 buffers, of 0.6 and 2.0 M of NaCl. Two consecutive gradient of a 0.0075 M/min and 0.0375 M/min, respectively were used. Fractions of the adsorption and elution stages were analyzed by gel electrophoresis and the purity of the pVAX1-NH36 were determined by hydrophobic HPLC.

Results. Figure 1 shows the chromatogram of the purification of pVAX1-NH36 by AE-Chromatography. A remarkable separation can be observed between the second and third peaks, corresponding to RNA and pDNA respectively. Gel electrophoresis (Fig. 2), shows that the pDNA was completely retained in the column in the adsorption stage (lines 4 to 6). A great amount of RNA elutes during the washing (7-11) and first elution gradient (12-15 and 18) steps. The purified pVAX1-NH36 was obtained in the second gradient (19-25). The fraction collected at 26 min had a relation A_{260}/A_{280} = 1.93 and the HPLC analysis showed a purity near to 93% and a concentration of 4.635 ± 1.128 μg/mL.

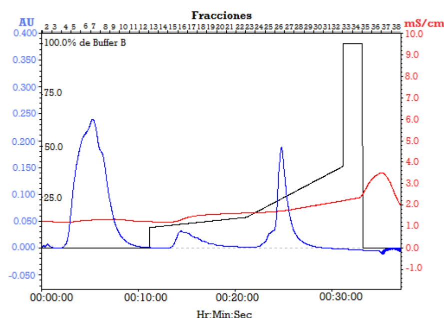

Fig. 1. AE-Chromatogrphy of a clarified lysate containing pVAX1-NH36 recovered after TFF. The blue line corresponds to the absorbance (AU), the red line conductivity (mS/cm) and the black line the applied gradients.

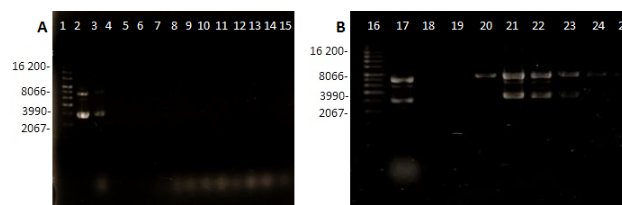

Fig.2. Electrophoresis from AE-Chromatography of a clarified lysate. Lines description: 1 and 16, molecular marker; 2, pVAX1-NH36 purified with a kit; 3 and 17, lysate solution; 4-6 adsorption stage; 12-15 and 18, fractions recovered during the first gradient; 19-25, fractions corresponding to the second gradient.

Conclusions. Plasmid recovered by tangential flow filtration was further purified using a tandem elution gradients in perfusion AE chromatography.

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