CAPILLARY ELECTROPHORETIC SEPARATION OF SEVERAL RECOMBINANT PROTEINS.

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Introduction: In recent years the zone capillary electrophoresis (CZE) has been in many cases the technique of choice for the analytical separation of proteins, peptides and complex mixtures.

Recombinant DNA technology has been successfully applied to the cloning and expression of human genes coding of proteins of pharmaceutical interest and have allowed the production of these therapeutic proteins. The development of these techniques is accompanied by the need of analysis methods to characterize these biologicals at different stages of the production. In control analysis several advantages, such as reduced animal requirements and testing time, are seen in the replacements of bioassay with physicochemical methodologies.

Optimize CZE separations conditions were development and used to analyze recombinant proteins produced in Genetic Engeenering Center.

Methodology: Recombinant proteins are produced at Genetic Engeering Biotechnology Center, Cuba.

Severals recombinant were studied: Surface antigen from hepatitis B virus, alpha, and gamma interpheron, streptokinase, P64k (protein from Neisseria meningitidis), P64k-EGF (fusion protein between P64k and grow epidermal factor) and TAB9 (multyepitopic polypeptide against different isolates from HIV virus). We used fused-silica capillaries with an I.D. of 50 m and an O.D of 363 m were obtained from Supelco Park (Bellefonte, PA, USA).The detection was performed by wavelength of 214 or 200 nm, respectively.

All separations were performed using Hewlett-Packard Instruments, USA and the data were collected and analyzed using the Hewlett-Packard data analysis software version Rev.A.06.03.509.

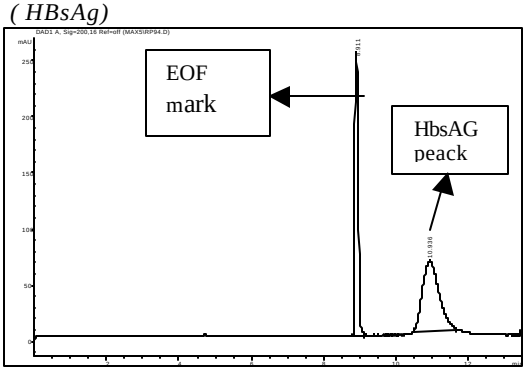
The CZE was carried out with the cathode placed at the detector end of the capillary, using 25 kV, at 25^{o}C. Before each run the capillary was rinsed with NaOH for 2 minutes followed with water 2 minutes and separating buffer 5 minutes.

The samples were hydrodynamically injected into a 58.5 cm (50 cm to the detector) fused silica capillary of I.D. 50 m by 35 mbar for 5 second.

To check EOF, 0.25% solution of dimethylsulfoxide (DMSO) we used.

Results and Discussion: We studied the behavior of theses protein in different background of electrolytes. The concentration of the proteins varied from 0.5mg/mL to 10 mg/mL. One example is showing in Figure 1.

Fig.1: Study in CZE of the surface antigen from hepatitis B virus (HBsAg)



Separation conditions: phosphate buffer 200 mM pH 7.
Migration time of the EOF peak: 9.91min.
Mobility: 19.7 x 10-9 m2V-1s-1
Migration time of the HBsAg: 10.9 min.
Mobility: -1.9 x 10-9 m2V-1s-1

Conclusions: We obtained separation conditions for all recombinant proteins studied. In majorities of the proteins was at high ionic strength. We calculated the mobilities of theses proteins in background electrolytes applicable for their separation.

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