

COUPLING OF PROTEINS TO NANODIAMONDS USED AS PSEUDO-STATIONARY PHASES FOR THE DETERMINATION OF NEUROLOGICAL DISEASE BIOMARKERS

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In the last two decades, significant interest has been reported on the use of nanometer sized diamond particles (NDs). These nanoparticles have attracted the interest of the nanoscience and nanotechnology communities, because of the potential use in the manufacturing of bright, low voltage (cold) cathodes and light emitters nanodiamond films. Furthermore, chemical modifications of the surface of the nanoparticles are becoming of significant value for the synthesis of bionanoconjugates.

Glucose transporter type 1 (Glut1) deficiency syndrome is a rare genetic metabolic disorder characterized by deficiency of a protein that is required for glucose to cross the blood-brain barrier. In children it affects the nervous system and it can be manifested with a variety of neurological symptoms. The most common symptom is seizures, which usually begin within the first few months of life. However, the symptoms and severity of Glut1 deficiency syndrome can vary substantially from one person to another. If diagnosed in the early stage of the disease formation, it can be of help to improve the quality of life of patients.

The aim of this study was to apply NDs as an immunosupport for the determination of Glut1 in a red cells lysate using an affinity capillary electrochromatography technique. The Glut1 antibodies were attached to the NDs and the bionanoconjugate was added to the background electrolyte (BGE) as a pseudostationary phase (PSP).

A Beckman P/ACE MDQ capillary electrophoresis instrument was used. The dimensions of the capillary were 50 μm i.d. and 15 cm total length, and the pseudostationary phase system consisted of sodium phosphate 40 mmol/L, pH 7.4, and 7% NDs-Glut1 antibodies covalently bonded by the carboxylated groups present in the nanodiamonds and the amine groups present in the antibodies.

The proposed method was successfully employed for the detection of normal Glut1 in a red cells lysate. The use of NDs-Glut1 antibodies as pseudo stationary phases in nanoelectrokinetic chromatography (nanoEKC) allows for the determination of the protein of interest with minimal treatment of the samples.