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DEVELOPMENT OF FERRITIN ELECTROCHEMICAL IMMUNOSENSOR BASED ON CPE MODIFICATION

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Abstract

Ferritin is a major intracellular iron storage protein present in all cells, tissues and tissue fluids of the organism. Low ferritin levels result in lower iron concentrations which is directly involved with anemia. Elevated levels of ferritin, or hyperferritinemia, indicate the presence of viruses and bacteria into the body. Clinical observations on COVID-19 patients have reported cases accompanied by elevated levels of ferritin in blood. The aim of this research was to develop a new voltametric immunosensor for determination of ferritin based on the principles of biological recognition, antibody-antigen reaction combined with nanotechnology and the advantages of electrochemical detection strategies.

Carbon paste electrode (CPE) modified with grain natural material, characterized as titanium magnetite is used as substrate for immunosensor. The immobilization of ferritine antibody (FeAb) can be effectively improved by using a thin film of surfactant, trimethyl-tetradecylammonium chloride (TTDC), onto the CPE substrate. The modification procedure of the immunosensor is characterized by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The effect of FeAb incubation time and the FeAb-ferritine reaction kinetic are explored to provide optimum analytical performance. The quantitative determination of ferritine is based on the change in DPV response before and after antibody-antigen reaction. All measurements are done in pH = 7 phosphate buffer saline (PBS) at room temperature. Calibration method was based on the reduction of the DPV peak (%) in relation to the ferritin concentration.

The time required for the immobilization of FeAb was studied, which resulted in 60 minutes, as well as the equilibrium time of the FeAb-ferritin reaction, which resulted in 30 minutes. The linear range resulted within the interval 0.05 - 0.5 mg/L ferritin (R2 = 0.9947). The recovery of ferritin addition in real sample matrix resulted from 87% to 125%. The specificity of FeAb-ferritin reaction evaluated in terms of binding constant, resulted in the order of 10 - 9 L/mol indicating a specific antibody-antigen reaction.

Based on the values of affinity constants calculated in each case the quantification of ferritin with the studied sensors is based on the specific antiferritin-ferritin bond. The use of surfactant layer (TTDC), improves the process of antiferritin immobilization, which affects the increase of sensitivity and improve the analytical performance of the imunosensor.

***Key words**: Ferritin, Imunosensor, Carbon paste, Surfactant, Modification.*