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DEVELOPMENT, CHARACTERIZATION AND APPLICATION OF MULTI-DETECTOR FLOW INJECTION SYSTEMS BASED ON A PARALLEL FLOW CONFIGURATION FOR SIMULTANEOUS DETERMINATION OF FREE AND BOUND SIALIC ACID

by

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Abstract

Oxidase enzymes have been used successfully in the analysis of several biochemical species such as glucose, lactate, pyruvate, creatine, creatinine, amino acids, cholesterol and sialic acid, by generating - in a single or consecutive reaction - hydrogen peroxide (H_2O_2) as a signal molecule which is commonly detected amperometrically. Some approaches have been also reported to use more than one enzyme in a given system to achieve multi-analyte determination. This study aims to evaluate a novel approach for n -analyte determination based on n -enzyme reactors (each is loaded with one or more oxidase enzyme) as well as n -1 catalase immobilized reactor. Therefore, a total of $2n-1$ immobilized enzyme reactors (IERs) will be used in the direct determination of n -analytes in a given sample injection using a flow injection (FI) system equipped with n -channel amperometric detector. Single sample injection would result in a tandem of n -peaks, the height of which correspond to the concentration of present n -analytes, respectively. Developing the needed multi-channel amperometric detector with two demanding requirements, i.e., small dead volume and high-pressure rating (≥ 40 bar) was also aim of the present study. Two 4-channel amperometric flow cells for the screen printed electrodes (SPE) and conventionally sized electrodes, were carefully designed, fabricated and evaluated. Both channels proved suitable for 4-channel amperometric detection of H_2O_2 , however the cell of the conventionally sized electrodes was used in evaluating the proposed analytical scheme based on IERs because of the stability and reusability of conventional platinum electrodes. Finally, three IERs loaded with glucose oxidase, lactate oxidase and pyruvate oxidase, respectively as well as two identical IERs loaded with catalase were successfully to validate the proposed analytical system. The obtained response showed three FIA peaks for samples containing glucose, lactate and pyruvate without any sign of channel cross-talk. The described system is a new effective way to achieve multi-analyte determination by flow injection analysis. Special benefit will be for those important biochemical such as bound-sialic acid which is converted into H_2O_2 by three enzymes which makes the analysis susceptible to interference from indigenous related species such as free sialic acid and pyruvate.

Keywords: Multi-analyte determination, Amperometry, Flow-Cell, Flow-Injection Analysis, Immobilized Enzyme Reactors, Sialic acid.