ANALYTICAL CAPABILITIES OF VARIOUS APPROACHES TO ELECTROPHORETIC DETERMINATION OF MONO- AND DISACCHARIDES

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Carbohydrates play a significant role in metabolic processes and their determination in complex biological matrices is applicable for solving diagnostic and food manufacturing problems. Capillary electrophoresis (CE) is an analytical method used for the separation of carbohydrates as well as other analytes with high efficiency, and separation selectivity. Besides, the method provides possibilities for lowering the limit of detection (LOD) for selected analytes using in-capillary sample concentration techniques and intracapillary derivatization. Still, approaches for the electrophoretic detection of carbohydrates aim to overcome the following limitations: absence of chromophore groups, high polarity, and low values of dissociation constants.

Thus, it is possible to determine carbohydrates in the indirect detection mode using the absorbing “probe” ion introduced into the background electrolyte (BGE). The absorbing “probe” selection considers its stability in the BGE, high molar absorptivity, and mobility close to the mobility of analytes to improve separation efficiency. Indirect detection was used for the determination of sugars with the following BGE: 2.5 mM acridoneacetic acid as an absorbing “probe”, 75 mM KOH, 0.5 mM cetyltrimethylammonium bromide, and 5% (vol.) methanol. Organic acids and carbohydrates migrated as separate groups of analytes, and the separation of seven carbohydrates was reached. The method was applied for baby food analysis.

Common pre-analysis derivatization methods, which add chromophore group to the carbohydrate molecule, are reductive amination and condensation with 1-phenyl-3-methyl-5-pyrazolone (PMP). Reductive amination was performed using ethyl-p-aminobenzoate (ABEE), and separation conditions for the derivatives were selected. Derivatives were separated in the CZE and the MEKC modes. Sodium dodecyl sulfate (SDS) was introduced into the BGE to form micelles that could enhance the separation of the derivatives through hydrophobic interactions. The MEKC mode was used for the separation due to its higher efficiency and the separation selectivity: the efficiency ranged from 408 to 654 thousand t.p., the selectivity factors were 2.1 – 2.4, and the LOD were 0.75 – 2.93 μg/mL. In-capillary sample preconcentration was performed using sweeping with the addition of micelles into the BGE without their addition into the sample. The sample enhancement factors reached ranged from 13.4 to 19.3. The capabilities of ionic liquids for the separation enhancement were tested as they were introduced into the BGE separately and along with SDS to form mixed micelles.

Derivatization conditions were optimized for analytes separately using the central composite design. Optimal conditions for the derivatization were determined for each analyte using response-surface methodology. Mutual optimization was performed with the desirability function, and experimental yields in the conditions selected complied with the predicted ones. The intracapillary derivatization procedure included the introduction of the sample, the reagent solution, and the BGE zone into the capillary with the following electrophoretic mixing. The SDS micelles were added into the reagent solution to provide its electrophoretic mobility.

Condensation of sugars with PMP was used for the determination of reducing carbohydrates and the derivatives were separated in the CZE mode. The separation efficiency was 200 – 260 thousand t.p., the selectivity factors ranged from 1.0 to 3.4, and LOD was from 0.30 to 0.49 μg/mL. The use of large-volume sample stacking enabled the lowering of LOD to 0.10 – 0.16 μg/ml. Linearity of both derivatization methods was established in the range of concentrations from 10 to 250 μg/mL. Both derivatization methods were adapted to the analysis of baby food.

Therefore, indirect detection and two derivatization methods were used for the electrophoretic determination of carbohydrates. Analytical characteristics of each method were compared and all were applied for the analysis of baby food samples. Besides, the capabilities of in-capillary sample preconcentration and intracapillary derivatization for reductive amination were investigated. The MEKC mode for the reductive amination derivatives demonstrated high efficiency, separation selectivity, and low LOD among the tested ones.

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