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Determination of Inorganic Phosphate by the Use of Immobilized Enzymes in a FIA System*

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Inorganic phosphate was fluorometrically determined in the FIA system with a column containing co-immobilized enzymes for successive reactions to generate NADPH from inorganic phosphate. The enzymes used were sucrose phosphorylase, phosphoglucomutase and glucose-6-phosphate dehydrogenase, and they were co-immobilized on aminopropyl glass. Assay conditions such as flow rate of a carrier solution and column temperature were investigated for optimization. Several buffers of different pHs were examined as a carrier solution to find Tris buffer (pH 8.0) most favorable. Under the conditions, the calibration curve for the determination of inorganic phosphate (50 μ l injection) showed a linear relationship in the concentration range of 0.2–100 μ M. The detection limit was 0.1 μ M, that is, 5 pmol. The relative standard deviation of the peak area for 10 μ M sample was 4.1% (n = 5). The analysis of a sample completed in 10 min. This method was applied to the determination of phosphorus content in the calf thymus DNA. The content obtained was coincident with that obtained by the molybdenum blue method.

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