

**NOVEL TRENDS IN ANALYTICAL SCIENCES: CAPILLARY ELECTROPHORESIS AND MICROCHIP CAPILLARY ELECTROPHORESIS**

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**ABSTRACT**

Recently, a demand for rapid, cost-effective, and environmentally friendly analytical methods has increased. These approaches can be achieved with electrically driven separation methods known as “capillary electrophoresis (CE)” due to short analysis time, low solvent and sample consumption, and high separation efficiency. Additionally, the miniaturization system, “microchip CE”, enables promise for higher sample throughput, lower solvent and sample consumption, and consideration as a portable instrument.

This work purposed to evaluate the merits of these techniques in chemical and biological fields towards green analytical chemistry. The CE method for the simultaneous analysis of nicotine, cotinine, nicotinamide, and nicotinic acid was established using triprolidine as an internal standard. The optimized condition was achieved in 25 mM sodium dihydrogen phosphate (pH 2.1) using a capillary with a  $L_{total}$  of 64.5 cm, 50  $\mu$ m i.d. (extended path length), injection at 50 mbar for 10 s, and the applied voltage of 30 kV, and a baseline separation at 10 min. The validated method was applied for the determination of nicotine, cotinine (in a stress test of nicotine gum), nicotinamide, and nicotinic acid in pharmaceutical formulations with the results found within USP limit.

Furthermore, the performance of commercial microchip CE with a red laser induced fluorescence detection and its chips were evaluated. Simple and rapid chip-based non-aqueous CE separation of several structurally related basic dyes (*e.g.* methylene blue, toluidine blue, nile blue, and brilliant cresyl blue) was achieved in ~ 40 s in 80 mM  $\text{NH}_4\text{OAc}$ , 870 mM acetic acid in DMSO with a separation length of 14 mm and injection/separation voltages of 1,400/1,500 V. Then, single cell analysis of nile blue stained *E. coli*, *B. subtilis*, *M. luteus*, *S. aureus*, *C. albicans*, and *L. fungicola* was achieved in ~ 20 s in 1 mg/mL CTAB in 1 mM Tris/0.33 mM citric acid, pH 7.0, using 5 mg/mL SB3-10 as a blocking agent and injection/separation voltages of -1,000/-1,000 V providing S/N of 26.2 – 34.0 with % RSD within 2.45%. Separations of the bacteria and fungus mixture containing *E. coli*, *S. aureus* and *C. albicans* were re-optimized and achieved in 3.94 mM Tris, 0.56 mM boric acid and 0.013 mM  $\text{Na}_2\text{EDTA}$  pH 10.5, containing 0.025% PEO with injection/separation voltages of 1,000/1,000 V. Additionally, the optimal condition was successfully applied for the separation of Gram positive bacteria (*i.e.* *B. subtilis*, *M. luteus*, and *S. aureus*).

**KEY WORDS:** CAPILLARY ELECTROPHORESIS/ PYRIDINE/ MICROCHIP  
CAPILLARY ELECTROPHORESIS/ FLUORESCENT DYE/  
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