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Review

Native fluorescence detection of biomolecular and pharmaceutical compounds in capillary electrophoresis: Detector designs, performance and applications: A review

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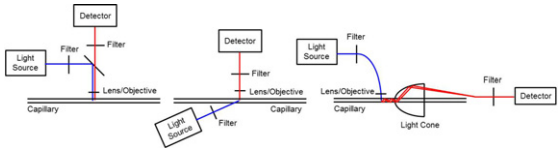
HIGHLIGHTS

- The use of native fluorescence detection in capillary electrophoresis is reviewed.
- Various detector designs are discussed, and their performances are evaluated.
- Specific attention is devoted to fluorescence detection in microfluidic systems.
- Applications of biomolecular and pharmaceutical compound analysis are described.

GRAPHICAL ABSTRACT



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ABSTRACT

This review treats the coupling of capillary electrophoresis (CE) with fluorescence detection (Flu) for the analysis of natively fluorescent biomolecular and pharmaceutical compounds. CE–Flu combines the excellent separation efficiency of CE with the high selectivity and sensitivity of Flu. In CE–Flu, an appropriate design of the fluorescence detection cell is very important in order to achieve efficient analyte excitation in and emission light collection from the small cylindrically-shaped detection volume. Therefore, due attention is paid to the various optical detection designs used for CE–Flu, including the applied excitation sources and emission light detectors. Special attention is devoted to wavelength-resolved Flu and to sensitivity issues. Furthermore, he specific requirements for fluorescence detection in microfluidic systems (*i.e.* chip-based electrophoresis) are discussed. Subsequently, an overview of described applications of CE–Flu for the analysis of natively fluorescent biomolecules and drugs is presented in extensive tables, treating amino acids, peptides, proteins, bioactive compounds, flavins, pharmaceuticals and also single cell analysis. The tables provide information on analyte nature, sample matrix, optical detection aspects, CE mode and limits of detection. A selection of descriptive applications is discussed in detail to illustrate the potential of native fluorescence detection in CE. It is concluded that CE–Flu is a powerful tool for biomolecular and pharmaceutical analysis, and provides good opportunities for use in lab-on-chip devices.

Contents

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Bregje J. de Kort obtained her MSc degree in Molecular Sciences in 2007 from Wageningen University, The Netherlands. There she did a research major studying a folding intermediate of the protein apoflavodoxin. From 2007 she works at the Biomolecular Analysis group at Utrecht University, where she obtained her PhD in June 2012. Her thesis research focused on the development of wavelength-resolved fluorescence detection for capillary electrophoresis (CE) as a novel tool for protein characterization. Her current research interests include (biopharmaceutical) protein characterization and protein (un)folding as studied by CE combined with fluorescence and mass spectrometry.

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1. Introduction

Over the last 20 years, capillary electrophoresis (CE) has developed into an established technique for the analysis of pharmaceutical and biomolecular compounds [1–3]. It offers attractive features such as efficient separation, short analysis times and requirement of only minute amounts of sample. Automated CE instrumentation is commercially available from several manufacturers. Development of microfluidic systems for chip-based electrophoresis has further reduced analysis times and sample requirement [4].

CE comprises several separation modes, like capillary zone electrophoresis (CZE), capillary isoelectric focussing (cIEF), (micellar) electrokinetic capillary chromatography ((M)EKC), capillary gel electrophoresis (CGE) and non-aqueous capillary electrophoresis (NACE), each of which provides a distinct selectivity for a variety of drugs and biomolecules [3,5]. CZE is the most common separation mode in CE providing a separation mechanism based on analyte charge-to-size ratio. In cIEF, analytes can be separated based on their isoelectric point, whereas in CGE a sieving gel or polymer network is used to yield size-based separations. (M)EKC, being a hybrid of electrophoresis and chromatography, allows separation of neutral as well as charged compounds, and enables chiral separations. NACE is performed in non-aqueous background electrolytes (BGEs) providing unique resolution and permitting separation of basic or acidic compounds with a low solubility in water. Proper choice of the separation mode thus allows analysis of a wide range of compounds, such as amino acids, peptides, proteins, drugs and DNA fragments [3].

Detection in CE is usually carried out with on-capillary UV absorbance at low UV wavelengths (200–220 nm). UV absorbance arises mostly from π–π^* electronic transitions. This makes UV absorbance widely applicable to a broad range of molecules. However, the small inner diameter of the CE capillary provides a short optical path length for UV absorbance, leading to relatively poor concentration sensitivities. Moreover, detection selectivity is quite low since many compounds absorb in the low UV region. Absorbance from sample and/or BGE constituents can interfere with analyte detection, and result in (increased) background signals or instable baselines.

Some molecules emit fluorescence after excitation with light of an appropriate wavelength. For these compounds, detection of their native (*i.e.* intrinsic) fluorescence emission might be an interesting alternative for UV absorbance detection [6]. Several classes of biomolecular compounds exhibit fluorescence, like aromatic amino acids and peptides and proteins containing those amino acids, and biologically active compounds derived from these amino acids (like catecholamines and tryptamines). Also flavins and nicotinamide-based cofactors show native fluorescence, which can be used, for instance, to indirectly monitor enzyme kinetics [7]. Several pharmaceutical compounds containing aromatic structures, such as naproxen, propranolol, doxorubicin, salicyclic acid and several