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Detection in Capillary Electrophoresis – An Introduction

Gerhardus de Jong

Capillary electrophoresis (CE) has been developed into a strong analytical technique. Separation is based on charge-to-mass ratio, and high efficiencies can be obtained with short analysis times. In principle, CE is suitable for charged compounds, but by using micelles in the background electrolyte (micellar electrokinetic chromatography, MEKC), neutral compounds can also be separated. Other additives can increase the selectivity of CE, for example, cyclodextrins for chiral separations. The consumption of solvents is small as flow rates are very low and mostly aqueous buffers are used. This latter aspect also means that the technique is biocompatible and is well suited for the analysis of intact proteins. Next to capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF) and capillary gel electrophoresis (CGE) are powerful for the analysis of biopolymers. The reproducibility and robustness of CE are often less than those of liquid chromatography (LC) and gas chromatography, but in the past years, this has been improved by reliable injection techniques and more stable electroosmotic flows. Only small sample volumes are needed, which is favorable for some applications. However, the injection of low volumes is a disadvantage for the concentration sensitivity. This can be compensated by on-line electrokinetic and chromatographic preconcentration of relatively large volumes [1, 2]. Moreover, sensitive detection can decrease the detection limits, which is important for trace-level analysis.

The detection volume should be small, and an efficient combination (or even integration) of the separation capillary and the detector is required. On-capillary ultraviolet (UV) and especially diode-array detection is mostly used. Fluorescence is another optical system, and high sensitivity can be obtained by fluorescence detection in CE. However, derivatization is often necessary for attachment of a fluorophore to the analytes. Electrochemical detection has also been developed for CE and can be divided based on three principles: potentiometric, amperometric, and conductometric. Today, potentiometric detection and amperometric detection are rarely used. In early CE work, conductivity detection was the standard approach, and this universal detector is still applied for compounds that are difficult to detect by UV absorption. Coupling of CE and mass spectrometry (MS) is important as MS is sensitive and selective. Moreover, it can provide structure

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information and identification of unknown compounds. Efficient interfacing has been realized and CE-MS is now well established. The design and potential of the main detection modes in CE will be shortly described in this introductory chapter.

1.1 UV Absorption

UV absorption is by far the most common detection mode in CE, and on-capillary detection is a part of commercial instruments. It is a universal principle as the use of fused-silica capillaries and aqueous buffers allows detection wavelengths below 200 nm up to the visible region of the spectrum. The use of low wavelengths offers a significant gain in sensitivity and wide applicability. The detector volume is very small, which means that band broadening is prevented. However, the design is critical and the optical path length is equal to the capillary diameter, which limits the sensitivity. Moreover, the linear detection range is limited due to the small size and the curvature of the capillary. The bubble cell, the *Z*-shape cell, and similar flow cells have been developed to increase the optical path length, but peak broadening may occur and the cells are not often employed.

For compounds that do not exhibit UV absorption, indirect detection can be applied. An absorbing co-ion is added to the background electrolyte (BGE), and this is displaced by the analyte. At the position of the analyte, a negative peak will appear. The displacement depends on the charges of the probe and the analytes and on their mobilities. Each fluctuation in the probe concentration is detected as noise. In principle, indirect UV detection is universal but optimization is rather complex [3]. The choice of the monitoring ion and other components of the BGE needs special attention. Examples of analytes are inorganic and simple organic ions and sugars. Typical detection limits are in the micromolar range. For detection of anions, monitoring ions such as chromate, benzoate, and phthalate can be used. For cations, for example, imidazole and pyridine are added to the BGE.

1.2 Fluorescence

Fluorescence is very sensitive, especially if a laser is used as excitation source. Excitation light should be focused on a very small detection volume with curved walls of the capillary. Furthermore, analyte emission should be effectively collected from the same volume. The inner and outer surfaces, which refract the excitation and emission light, cause scatter, which in turn can induce significant background noise. The fluorescence is emitted in all directions and only a small part is collected. Therefore, proper attention should be paid to the design of the optical configuration to allow sensitive detection in CE [4]. The analytes can be detected on-column (i.e., inside the capillary) or off-column. The determination of attomol and sub-ng ml^{-1} levels has often been demonstrated. Because of its small

sample requirements, CE with laser-induced fluorescence is an excellent tool for single-cell analysis [5].

Many molecules do not exhibit fluorescence, and therefore, this detection mode is also selective. On the other side, a wide range of derivatization reagents have been developed to confer fluorescent properties to compounds that are intrinsically not fluorescent [6, 7, 8]. Various reagents are commercially available and can be easily applied. Recently much attention has also been paid to the possibilities of in-line derivatization (8). The appropriate excitation wavelength should be chosen for the analyte(s) of interest. Excitation sources that allow flexible wavelength selection are xenon, mercury–xenon, and deuterium lamps. By the use of a grating or filter, a suitable wavelength can be selected. Lasers emit monochromatic light with a high intensity and directionality. This facilitates focusing of the light onto the capillary, which is one of the main reasons for laser-induced fluorescence detection in CE. Unfortunately, there are only a few laser lines available in the deep-UV region. More recently, light-emitting diodes (LEDs) have become an attractive alternative for lasers as an excitation source due to their small dimensions, stable output, and low costs [9]. LEDs are semiconductive light sources and the wavelength is determined by the semiconductor material. Commercial LEDs are available from the deep-UV to near-IR region.

Detection of the emission light is most often performed using a photomultiplier tube in combination with a filter. In order to obtain spectral information, an imaging detector, for instance, a charge-coupled device, is required. When this is combined with a spectrograph, emission spectra of analytes can be monitored in the wavelength-resolved detection mode [10].

1.3 Conductivity

In the early stage of CE, conductivity was often used for detection. With the introduction of fused-silica capillaries, this was replaced by UV and fluorescence detection. However, there are still some strong points for conductivity detection in CE. The technique is universal and suitable for compounds such as inorganic ions. Contactless conductivity detection (CCD) is a very useful detection mode as electrodes do not contact the BGE in the on-capillary design [11, 12]. Two stainless-steel tubes mounted around the capillary act as the so-called actuator electrode and pick-up electrode. A capacitive transition is generated between the actuator electrode and the liquid inside the capillary. After passing through the gap between the electrodes, a second capacitive transition between the electrolyte and the pick-up takes place, and if the conductance changes by analytes, this will be measured by the pick-up electrode. The difference in conductance between the analyte zone and the BGE should be as high as possible. Moreover, the conductivity of the BGE is very important and should not be too high in order to prevent high noise and not be too low as this causes electrodispersion. A compromise is the use of amphoteric buffers at relatively high concentrations. Limits

of detection for cations and anions in the ng ml^{-1} region have been obtained. CE-CCD has also been used for the separation and detection of saccharides and underivatized amino acids. The system has successfully been applied for the analysis of biological samples. CCD can easily be coupled to other detectors and is well suited for microfluidics CE [13].

1.4 Mass Spectrometry

MS is attractive for detection in CE as efficient separation is coupled with sensitive and selective detection of small and large molecules. Simultaneously, MS and MS/MS can be used for identification of compounds. Moreover, compounds that coelute in CE may easily be distinguished in MS. The development of reliable CE-MS took a rather long time. As in LC, the challenge is to combine a liquid-phase separation technique with a vacuum detection technique. Especially, this is difficult for MEKC, CIEF, and CGE as the run buffer contains high concentrations of nonvolatile components. However, an advantage of CE is the use of very low flow rates (nl min^{-1}). Furthermore, electrospray ionization (ESI) is well suited to ionize polar and charged substances separated by CE. The complexity for the interfacing is that both CE and ESI are based on an electrical field and the fields should be combined. Several approaches have been described in the literature, and after many years of development and optimization, CZE-MS can now be used in routine [14]. Recently, a collaborative study on the robustness of CE-MS for peptide mapping has been presented [15]. The results demonstrate that CE-MS is robust enough to allow method transfer across multiple laboratories. This is an important step for the technological maturity of CE, also with respect to LC-MS, which is a very strong analysis technique in various areas. The high complementarity of CE-MS has been demonstrated for metabolic and proteomic profiling [16, 17].

Principles and applications of CE–MS are described in this book. The next chapter will show the fundamental aspects of CE–MS and emphasize the interfaces. And the subsequent chapter will highlight the sheath-liquid interface, still the most important coupling between CE and MS. Separate chapters will describe the developments in microchip CE-MS and the potential of the on-line combination of sample preconcentration and CE-MS. The other chapters will focus on different application fields and show the wide applicability of CE-MS. Important technological information and many illustrative figures are presented. Both basic aspects and the state of the art of CE-MS are shown.

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