11
Detectors in Capillary Electrophoresis

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11.1
Introduction

Substantial demands are placed on detection systems for CE. The internal diameters of the electrophoretic capillaries vary between 10 and 100 μm. These small spatial dimensions are connected with small sample volumes injected into the capillaries (tenths to tens of nanolitres) and frequently with small analyte concentrations in the sample. For these reasons, high sensitivity and miniaturization of detection systems is required. In addition, the detection cells must be designed so as not to contribute to additional broadening of the zones of separated substances. The most sensitive contemporary detector is based on laser-induced fluorescence (LIF). In most cases, derivatization of the analyte is necessary for these detector systems and the apparatus is relatively complicated; thus, universal detectors based on various physical–chemical principles are being developed and used. On-capillary detectors that detect the analyte directly in the capillary are preferred from the viewpoint of the effect on the separation efficiency. Off-capillary detectors detect the analyte leaving the end of the separation capillary and generally require exact adjustment of the detection element in relation to the end of the capillary. Very effective detection systems are multi-detectors combining several detection systems that work on various physical–chemical principles.

The great majority of detection systems described in professional publications have never left the chemical laboratory and a number of them have complicated working protocols or only very specific uses. In normal practice, on-capillary UV absorption, fluorescence, and conductivity (now mostly in the contactless mode) detectors are used, together with off-capillary amperometric detectors. Off-capillary detectors include mass-spectrometric detection, which will be discussed elsewhere in this chapter. The approximate limits of detection (LOD) of various detectors are listed in Table 11.1. The principles of the detectors used most frequently in CE are discussed in the next section; details can be found in the mentioned reviews and especially in the original literature to which the reviews refer.

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Table 11.1 Approximate detection limits of the commonest CE detectors.

Detector	LOD [mol]	LOD (mol/l)
Absorption UV – direct	10^{-12}–10^{-15}	10^{-6}
Absorption UV – indirect	10^{-12}–10^{-13}	10^{-5}–10^{-6}
Fluorescence[a)]	10^{-15}–10^{-20}	10^{-10}–10^{-11}
Amperometric	10^{-18}	10^{-7}–10^{-8}
Conductometric	10^{-16}	10^{-7}–10^{-8}
Electroluminescence	10^{-16}	10^{-6}
Mass spectrometric	10^{-17}	–

Note; From the data in Refs. [1–3].
[a)] LOD depends on the derivatization method.

11.2 Optical Detection

11.2.1 UV-Vis Photometric Detection

Spectrophotometric detection in the ultraviolet (UV) and visible (Vis) parts of the electromagnetic spectrum, that is, UV-Vis absorption, is the most frequently employed detection technique in CE and detectors based on this principle are a standard part of commercial electrophoretic instruments. Detection is performed in a short section of the fused silica separation capillary, from which the protective polyimide layer is removed (unless a capillary covered with a UV-transparent protective layer is used). Monochromatic radiation passes through this detection window with an input intensity of I_0, reaching with output intensity I the photodiode after passing through the capillary. Radiation reaching the photodiode is delimited by the slit whose dimensions define the optical detection cell. The length of the slit (longitudinal dimension) should not be greater than one-third of the normal peak width in CE, that is, about 2–5 mm, minimizing the effect of the detector on peak broadening. The width of the slit (radial dimension) depends on the internal diameter, i.d., of the capillary. This dimension prevents passage of the radiation through the walls of the capillary outside the solution, which might contribute to increasing the noise of the measured signal and negatively affect the slope and dynamic range of the calibration curve. It follows from optimization studies that the maximum signal/noise ratio is achieved at a slit width of 0.8–1.0 times the i.d. of the capillary. When this condition is fulfilled, the absorption of the radiation is determined primarily by the properties of the solution in the capillary and is measured as the absorbance A, defined by the equation

$$A = \log I_0/I. \tag{11.1}$$

The absorbance is an additive function and, according to the Lambert–Beer law,

$$A = L \cdot \Sigma\, \varepsilon_i \cdot c_i \tag{11.2}$$

is dependent on the length of the optical pathway, L, the molar absorption coefficient, ε_i, and the concentration, c_i, of all the substances present in the solution. The selectivity of the detection is determined by the wavelength of the employed radiation, which must be chosen according to the absorption spectrum of the monitored analyte.

11.2.1.1 Direct UV-Vis Detection

Direct UV-Vis detection measures the absorbance of the analyte in the zone passing through the capillary at the site of the detection window. For a capillary with circular cross section, the optical pathway is approximately equal to 0.8 times the i.d. of the capillary. The short optical pathway in normal capillaries is the main reason for the low sensitivity of UV-Vis detection, which is characterized for most analytes by an LOD at the level of 10^{-5}–10^{-6} M. The use of capillaries with large i.d. leads to an increase in the sensitivity but is associated with greater production of Joule heat, loss of the separation efficiency, and limitations on the use of a background electrolyte (BGE) with low conductivity.

Several technical procedures based on lengthening the optical pathway have been developed to improve the sensitivity of UV-Vis detection [4,5].

1) *Capillaries with extended path length* are commercially available (Figure 11.1a), that is, capillaries with widened i.d. at the detection site (also called *bubble cells*). The magnitude of the widening is between three and five times the i.d.. This approach has the advantage that the i.d. is widened

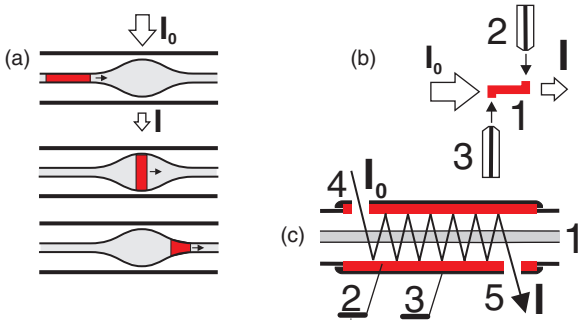

Figure 11.1 UV-Vis optical cells with extended path length. (a) Bubble cell and sharpening of the sample zone on passing through a bubble cell. (b) Z-cell: quartz block (1), ground end of the input (2), and output (3) capillaries. (c) Multireflection cell: separation capillary (1), silver coating (2), light protective coating (3), light inlet (4), and outlet aperture (5).

only locally and this does not reduce the overall resistivity of the capillary. In addition, the intensity of the electric field, *E*, is lower at the site of the widening than in the unwidened part of the capillary, which leads to slowing of the movement of the analyte zone and to its longitudinal shortening. Thus, the use of bubble cells does not lead to substantial peak broadening.

2) Another approach involves the use of optical cells with the shape of the letter Z, also called *high sensitivity cells* (Figure 11.1b), where the optical signal is recorded along the separation pathway. In commercially available high sensitivity cells, a Z-shaped separation channel is formed in a quartz block to which ground ends of the separation capillary and the short piece of an auxiliary capillary (connecting the separation part with the terminal vessel) are joined. In this way, the optical pathway can be lengthened to about 1 mm, but even 3 mm cells have been described. The increase in the sensitivity measured as the signal/noise ratio is, in fact, lower than would correspond to the geometric increase in the optical pathway, because not only the signal but also the noise increases with an increase in the optical pathway. As the optical signal is recorded in Z cells along the separation pathway, it is necessary to anticipate a loss in the separation efficiency and to employ this method for peaks with high resolution.
3) *Multireflection cells* [6] (Figure 11.1c) made in the laboratory are a technically interesting means of lengthening the optical pathway. A silver layer, acting as a reflecting surface, is formed on the outer surface of the fused silica capillary without the protective polyimide layer. It contains two transparent windows shifted mutually by approximately 1 mm; the remainder of the silver layer is shielded from the surrounding radiation by a black insulating layer. A beam from the laser source is brought through the entrance window, is reflected from the silver layer inside the capillary, and, after passing through the capillary a great many times, leaves through the other window and reaches the photodiode. This method enabled a 40-fold increase to be achieved in the sensitivity comparable to a *single pass cell*, with an LOD at the level of 10^{-7} M.

11.2.1.2 **Indirect Detection**

A considerable number of substances, such as inorganic ions, low molecular weight organic substances of the organic acid type, amines, saccharides, and most amino acids do not yield a strong signal in the UV-Vis part of the spectrum. The technique of indirect photometric detection was developed to detect these substances in CE [7]. In the optimization of the BGE composition [8], one of the components is a highly absorbing ion with the same charge sign as the analyte (coion) (see Table 11.2). During the separation, the absorbing BGE coions are forced out by the analyte ions that do not absorb according to the Kohlrausch regulation function (KRF). This leads to a lower concentration of the absorbing coions in the analyte zone, recorded as a negative peak or dip during passage through the detector. The difference in the coion concentration between the analyte zone and the surrounding BGE (Δc_C) depends not only on

Table 11.2 Survey of absorbing ions (probe) for indirect photometric detection and their application.

Probe	Wavelength (nm)	Analytes
Cations		
4-Aminopyridine	254	Li^+, Na^+, K^+, NH_4^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}
Tetrazolium violet	300	Chloroethyltrimethylammonium, tetrabutylammonium, tetrahexyl ammonium, and didodecyldimethylammonium
Methyl green	630	Li^+, Na^+, K^+, Cs^+, Ca^{2+}, Mg^{2+}
1,1-Diheptyl-4,4-bi-pyridinium hydroxide	280	Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Cd^{2+}, and NH_4^+
Chrysoidine	230	Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, and lanthanides
Anions		
p-Aminobenzoate	250, 264	Cl^-, SO_4^{2-}, NO_2^-, NO_3^-, formate, acetate, propionate, oxalate, and malonate
Phthalate	214, 230, 254	SO_4^{2-}, disulfide, tetrathionate, thiosulfate, phosphate, formate, acetate, propionate, oxalate, malonate, succinate, F^-, Cl^-, Br^-, NO_2^-, and NO_3^-
Pyromellitate	214, 254	SO_4^{2-}, disulfide, tetrathionate, thiosulfate, F^-, Cl^-, Br^-, NO_2^-, NO_3^-, acetate, citrate, lactate, and malate
5-Sulfosalicylate	214	Phosphate, carbonate, chlorate, and perchlorate
2,6-Naphthalene-dicarboxylate	240, 280	acetate, butyrate, propionate, carbonate, adipate, and glutarate
2,6-Pyridine-dicarboxylate	214	Br^-, I^-, NO_3^-, acetate, butyrate, citrate, formate, heptanoate, lactate, and malate

Note: Based on data from Ref. [7].

the analyte concentration (c_A) but according to KRF also on the mobility and concentration of all the participating ions. Hence, the replacement of the analyte ions by the coions does not generally occur in a concentration ratio of 1: 1; the final effect is described by the transfer ratio (TR).

$$TR = \Delta c_C / c_A. \tag{11.3}$$

TR must be maximized to achieve high sensitivity in indirect UV detection; this is connected with using low concentrated BGEs. When high concentrations of absorbing coions with high-absorption coefficients are used, the noise of the detector baseline is also high. To summarize, indirect UV detection is a universal detection technique in CE that can be used to detect all charged substances; on

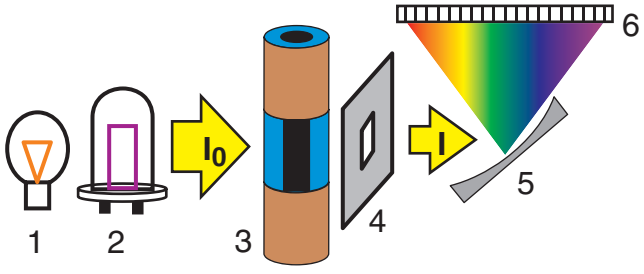

Figure 11.2 Basic scheme of a diode array detector. Tungsten lamp (1), deuterium lamp (2), separation capillary with detection window (3), optical slit (4), diffraction grating (5), and photodiode array (6).

the other hand, the attained sensitivity is 10–100 times lower compared to direct UV detection. The same detection cells as for direct UV-Vis detection are used. It should be added that contactless conductivity detection is considered an advantageous method for the detection of nonabsorbing ions (see Section 5.3.2).

11.2.1.3 Diode Array Detection

In classical UV-Vis detectors, the dispersion grid of the monochromator enabling selection of a suitable wavelength is placed in front of the optical cell. At present, classical types of detectors with a single detection element are replaced by diode array detectors (DADs) (Figure 11.2). In DAD, polychromatic radiation enters the detection cell and is dispersed by an optical grating after passing through the cell. The intensity of the dispersed radiation is recorded by the field of photodiodes where radiation in a certain wavelength interval falls on each diode. The signals from the photodiodes are gradually recorded and registered. The rate of signal recording is very rapid, where the signals from typically 1024 photodiodes forming the DAD can generally be obtained in less than 10 ms. In this way, the spectra of the substances in the individual separation zones are measured and can then be compared with the available recordings in the spectral library to determine the structure of the separated substances.

11.2.2 Fluorescence Detection

On absorption of radiation with frequency f_{ex}, the molecule passes from the ground electron state to an excited state with higher energy. If the molecule returns to the ground state through release of a part of the absorbed energy to its surroundings in the form of heat and emits the remainder as radiation with a frequency of $f_{em} < f_{ex}$, then fluorescence is involved. Fluorescence is an unusual phenomenon connected with a specific rigid structure of the molecule, which usually contains one or several aromatic rings. Of natural substances, fluorescence occurs in aromatic amino acids and their derived peptides and proteins,

Table 11.3 A survey of the most important fluorescence labeling reagents and their use in LIF.

Reagent	Molecules detected	λ_{ex} (nm)	λ_{em} (nm)	Laser
(native)	Peptides, proteins	260–280	305	Ar^+/KrF/Nd-YAG
FITC	Amino acids, peptides	442/488	550	Ar^+
OPA	Amino acids, amines	325/340	455	He-Cd
CBQCA	Amino acids, peptides	465	550	Ar^+/He-Cd
FQ	Immunoglobulins	442/488	550	Ar^+
Cy3 and Cy5	Nucleic acid	550/649	570/670	He-Ne, LEDs
NDA	Amino acids, peptides, and proteins	442	490	He-Cd, LEDs
EtBr	Nucleic acid	518	605	He-Ne
NBD-F	Amino sugars	470	530	Ar^+
ANTS	Oligosaccharides	325		He-Cd
TMR	Oligosaccharides	550	570	He-Ne
YOYO-1	Nucleic acid	491	509	He-Ne

Abbreviations: FITC, fluorescein isothiocyanate; OPA, *O*-phthalic aldehyde; CBQCA, 3-(4-carboxybenzoyl-2-quinoline-carboxyaldehyde; FQ, 5-furoylquinoline-3-carboxyaldehyde; Cy3, Cy5, cyanine dyes; NDA, naphthalene-2,3-dicarboxaldehyde; EtBr, ethidium bromide; NBD-F, fluoronitrobenzooxadiazole; ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid; TMR, tetramethylrhodamine; YOYO-1, benzoxazolium-4-quinolinium; YAG, yttrium-aluminum-garnet; λ_{ex} and λ_{em}, excitation and emission wavelength, respectively.

the metabolites of aromatic acids with the biological function of hormones and neurotransmitters, flavins, and nicotinamide-based cofactors permitting monitoring of enzyme kinetics. Among pharmaceutical preparations, these include, for example, naproxen, propanolol, doxorubicin, salicylic acid, and several drugs of abuse. For fluorescence detection of other substances, it is necessary to label them with a special labeling reagent, which bonds to a specific functional group. Table 11.3 gives a survey of the most important fluorescence labeling reagents and their use.

11.2.2.1 Laser-Induced Fluorescence Detection

An excitation source with high radiation intensity, which is focused on the detection window of the capillary and is not dispersed to the surroundings, is required to ensure high sensitivity of the fluorescence detection. Consequently, lasers yielding intense, highly monochromatic radiation focusing on an about 200 µm section of the capillary are used; the technique is called *laser-induced fluorescence* (LIF) [9–11]. In some fluorescence detector designs, the fluorescence is excited using a light-emitting diode (LED), where this detection system is called LEDIF.

In addition to good-quality excitation sources, effective focusing and collection of the emitted radiation on the detection element, usually photomultiplier tube (PMT), must be ensured, with separation from the excitation radiation by a

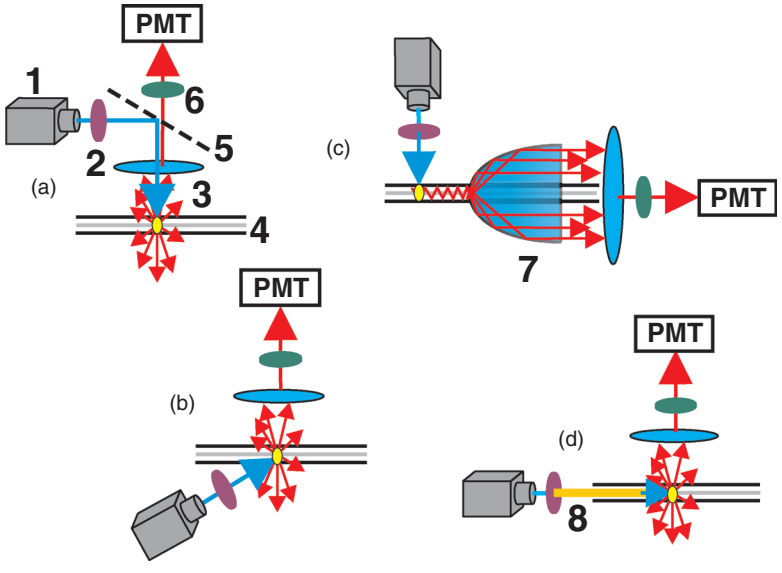

Figure 11.3 Basic scheme of a fluorescence detection cell. (a) Collinear arrangement: laser source (1), excitation filter (2), lens or microscope objective (3), separation capillary (4), dichroic mirror (5), emission filter (6), PMT; (b) right-angle arrangement; (c) arrangement with total internal reflection: light cone (7); (d) in-column excitation: light guide (8).

suitable detector design and optical filters. The fluorescence signal, S_{em}, passing through the detection window of the fused silica capillary can be theoretically predicted from the equation:

$$S_{em} = k \cdot I_0 \cdot V_{ex} \cdot \Phi_i \cdot \varepsilon_i \cdot c_i, \tag{11.4}$$

where constant k characterizes the effectiveness of capture of the emitted radiation, I_0 is the intensity of the excitation radiation in the detection cell, V_{ex} is the volume of the irradiated part of the capillary, Φ_i is the fluorescence quantum yield, ε_i is the molar absorptivity, and c_i is the analyte concentration.

The mechanical structure of fluorescence detectors for CE is more complicated than that of absorption detectors. Several types of detection cells have been proposed:

1) In the *collinear design*, the excitation source and detection PMT are located on the same side of the capillary (Figure 11.3a). The excitation radiation is focused and the emitted radiation captured using a single lens or microscope objective. The two rays are separated using a dichroic mirror, which reflects the excitation radiation from the source in the direction toward the detection site, while the emitted fluorescence radiation is allowed to pass to the PMT.
2) In a *right-angle arrangement* of the detection cells, the emitted radiation is collected in the direction perpendicular to the excitation ray (Figure 11.3b).

Two objectives of the microscope mutually rotated by 90° are used for focusing the excitation radiation and collection of the emitted radiation. This design minimizes undesirable interference of the excitation radiation with the emitted radiation and allows work with a pair of excitation laser sources and two detectors for *dual wavelength detection*.

3) The use of total reflection is an elegant approach for separating the excitation and emitted radiation (Figure 11.3c). The emitted radiation passes from the site of excitation along the separation capillary by total reflection on the quartz ($n = 1.458$)/air ($n = 1.000$) interface. A glycerol layer with high refractive index ($n = 1.473$) is located on the surface of the capillary about 1 cm from the site of excitation and the radiation leaves the capillary. Subsequently, the radiation is collected by a light cone fitted to the capillary surface and brought by a light-guide to the PMT. Spatial separation of the site of excitation from the site of collection of the emitted radiation substantially reduces the noise in the detector.
4) In addition to on-capillary geometry of fluorescence detectors, an in-capillary excitation technique has also been developed. Fluorescence is generated directly in the capillary space by excitation radiation brought along by an optical fiber fitted in the separation capillary (Figure 11.3d). The emitted radiation is recorded on the surface of the capillary using lenses or objectives and brought to the PMT. This excitation procedure substantially reduces undesirable scattering and reflection of excitation light by the wall toward the detector and simultaneously increases the effectiveness of the excitation.

11.2.2.2 Light Detection by a Charge-Coupled Device

Normally used PMT enables very sensitive detection of fluorescence radiation in a wide range of wavelengths (170–1200 nm). An imaging detector, for example, camera fitted with a charge-coupled device (CCD) [12] must be used to obtain information on the spectral composition of the emitted radiation or information on the spatial location of the fluorescing molecules. This method is used primarily in proteomics to depict developed bands of fluorescence-labeled proteins in 2D gels or blots. The same principle can be employed to create an image of the individual stationary zones of fluorescence-labeled proteins in capillary isoelectric focusing (CIEF). An optical fiber is placed at the entrance into the separation capillary (Figure 11.4) and conducts the excitation radiation of the laser. The radiation spreads in the internal space of the separation capillary on the basis of the principle of total reflection and fluorescence is generated in the stationary zones of the labeled proteins. The emitted radiation perpendicular to the surface of the capillary are detected using CCD camera. Processing of the signals from the camera enables depiction of the distribution of zones in a longer section of the capillary; this technique is known as *whole column imaging detection* [13].

CCD can also be used for spectral analysis in LIF [14], which is thus similar to DAD in absorption UV-Vis detection. The emitted radiation is collected by a

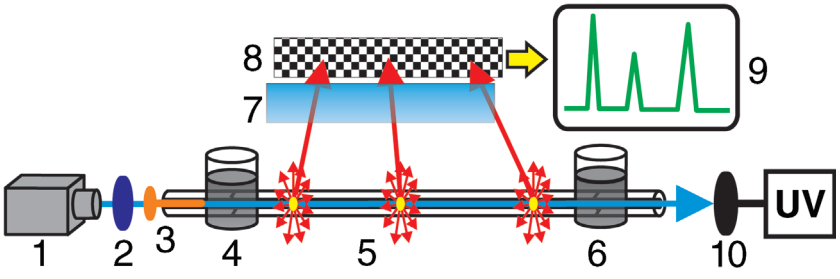

Figure 11.4 Basic scheme of whole column imaging detection for CIEF. Laser source (1), excitation filter (2), optical fiber (3), anode (4), separation capillary with focused zones (5), cathode (6), emission filter (7), charge-coupled device (8), reconstruction of electropherogram by PC (9), and photodiode for UV detection (10).

microscope objective and subsequently spectrally dispersed using a dispersion device. The intensity of the emitted radiation is detected for the individual wavelengths using CCD. The high effectiveness of this method is documented in the work [15] – the spectra obtained in the 260–710 nm range permitted identification of approxmately 30 substances derived from direct CE analysis of a single neuron.

11.3 Electrochemical Detection

The low sensitivity of UV detectors in the indirect UV detection mode, used for analytes not absorbing radiation, led to the development of alternative detection methods, including methods based on electrochemical principles. Almost all the basic principles have been utilized – potentiometry, amperometry, and conductometry. At present, only two of these are important, amperometric detection (AD), for which the general term electrochemical (EC) detection is often used, and conductometric detection, recently, primarily in the variant of capacitively coupled contactless conductivity detection (C^4D). While C^4D is less sensitive than AD, it has more universal use. Since C^4D was extended to general practice, contact conductometry has practically not been encountered in CE. Information on the instrumentation and application of electrochemical detection in CE (CE-EC) can be found in a great many review publications, recently, for example, [16–19].

11.3.1 Amperometric Detection

11.3.1.1 Principles of the Method and General Characteristics

AD is a sensitive method in which a constant potential, at which an electrochemically active substance is reduced or oxidized, is applied to the indicator

electrode. The Faradaic current, I, connected with these reactions is an analytical signal that can be directly correlated with the analyte concentration, c.

$$I \approx k \cdot c, \tag{11.5}$$

where k is a proportionality constant. AD is a selective method used for detecting only those substances that are oxidized or reduced at the applied potential. Oxidation is used more frequently to prevent interference from oxygen dissolved in the solution. The high detection sensitivity is a consequence of direct contact of the electrodes with the analyzed solution. On the other hand, this frequently leads to the loss of electrode activity as a consequence of adsorption of substances from the solution or the formation of films of the electrode reaction products. Pulse methods might be employed to eliminate the electrode "history", that is, to maintain constant electrode activity; in these methods, a combination of cleaning and measuring potential pulses is periodically applied to the electrode.

In addition to this general electrochemical problem, another problem is encountered in amperometric detection in CE in interference of the separation electric field with the signal from the detector. Three approaches are employed to eliminate this problem:

1) The off-capillary arrangement using a "decoupler" to bring the separation electric field from the capillary before the detector; the capillary is interrupted close to its end (e.g., by creating a crack in its wall, covered by a tube of ion-conducting polymer) and the interrupted site is placed in a reservoir with separation electrolyte, in which a grounding electrophoretic electrode is placed. The zones of the substance are brought through the remainder of the capillary by electro-osmotic flow (EOF) to its end, where the detection electrode is placed, Figure 11.5a (i).
2) The end-capillary arrangement, in which the end of the capillary is placed in a reservoir containing both the grounding electrophoretic and the detection electrode, Figure 11.5a (ii). The detection electrode is close to the outlet of the capillary and the detection conditions must be chosen so that the separation electric field leaving the capillary decreases substantially before it reaches the indication electrode. In general, in this kind of detection, it is recommended that capillaries with i.d. ≤ 25 μm are used, where the separation electric field decreases to a great extent almost immediately at the end of the capillary.
3) Another approach could be an electrically insulated potentiostat permitting placing the indication electrode inside the separation channel; the method was proposed for electrophoresis on a chip [20] but is not used much in practice.

11.3.1.2 **Detection Cell**

The small amounts of solution leaving the separation capillary and attempts to maintain high separation efficiency of the method require the use of detection

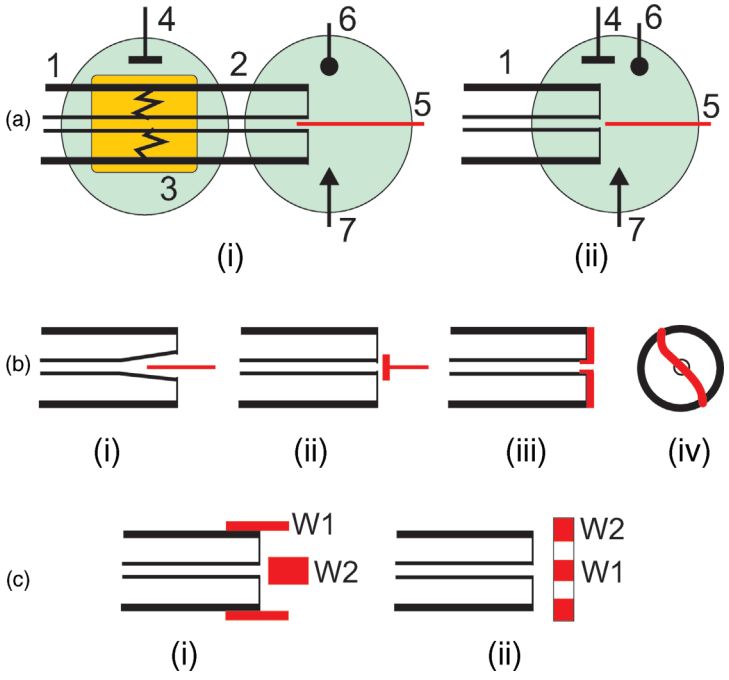

Figure 11.5 Basic scheme of an amperometric cell for CE-AD. (a) Geometric arrangement of an amperometric detection cell: (i) off-capillary arrangement with electrophoretic field decoupler; separation part of the capillary (1), detection part of the capillary (2), decoupler (3), grounding HV electrode (4), detection electrode (5), auxiliary electrode (6), and reference electrode (7); (ii) end-capillary arrangement. (b) Positions of the detection electrodes relative to the end of the capillary: (i) in-capillary arrangement, (ii) wall-jet, and (iii and iv) on-capillary arrangement. (c) Examples of arrangements of the indication electrodes, W, in dual AD–AD detection: (i) tube-wire, (ii) ring-disk. The reference and auxiliary electrodes are not depicted in Figure 11.5b and c.

electrodes with small dimensions, basically microelectrodes. Microelectrodes are suitable for AD not only because of their small dimensions but also because of their signal/noise ratio. The detection cell also includes auxiliary and reference electrodes, whose mutual positions in the cell are not usually critical. Detection electrodes can be in various positions relative to the outlet of the capillary:

1) *In-capillary*: an electrode with smaller external diameter (o.d.) than the i.d. of the capillary is slid into the capillary using a micromanipulator (at the end of the capillary, its i.d. can be widened to facilitate placing of the electrode) (Figure 11.5b (ii)).
2) *Wall-jet arrangement*: a usually disk-shaped electrode is placed close to the end of the capillary. In this case, the electrode has a greater diameter than the i.d. of the capillary so that it is easier to place it in relation to the outlet of the capillary (Figure 11.5b (ii)).

3) *On-capillary*: the electrode is an integral part of the capillary, that is, it is formed by metal-plating the end of the capillary or drawing a conductive fiber across the outlet of the capillary (Figure 11.5b (iii and iv).

The electrodes can be made from various materials – platinum, gold, or carbon of various forms. Electrodes based on carbon nanotubes (CNT) or boron-doped diamond (BDD) have been tested recently. For special purposes, for example, copper electrodes (detection of amino acids) and electrodes with chemically modified surfaces are used.

The selectivity or sensitivity of AD can be improved by simultaneous use of two indication electrodes, whose working parameters can be independently controlled by the potential, electrode material, or surface modification AD–AD detection can be used in several working modes – two of these are [21] as follows:

1) *Direct detection:* the electrodes are in a parallel arrangement and each is maintained at a different potential so that the substances leaving the separation column that are electrochemically active at different potentials can be selectively detected. Thus, substances leaving the capillary that are partly or completely not separated can be detected; an example of the electrode arrangement is given in Figure 11.5c (i).
2) *Indirect detection*: the electrodes are connected in series. At the first electrode of the series, an electrochemically active product, which reacts with the analyte, is generated from an auxiliary substance present in the separation buffer. The unreacted remainder of this product is detected at the second, downstream electrode; an example of the electrode arrangement is depicted in Figure 11.5c (ii). Thus, especially the selectivity of the detection is improved and it becomes possible to also detect electrochemically inactive substances. Indirect AD of electrochemically inactive substances is possible even using a single indication electrode. In this case, an electrochemically active substance is present in the BGE and is forced out of the zone by the analyte during the separation. Passage of the zone through the detector is then registered as a decrease in the registered current.

11.3.2 Contactless Conductometric Detection

11.3.2.1 Principles of the Method and General Characteristics

C^4D is a nonselective detection method based on measuring the electrical conductivity of the solution in the capillary. In contrast to the contact conductivity detection, the metal conductivity electrodes are placed on the outer wall of the separation capillary. The zones are detected inside the capillary (on-capillary), that is, the detector does not contribute to additional broadening of the analyte zones. The electrodes are not in contact with the analyzed solution so that there can be no problem related to the history of the electrodes, which can be a source of instability in the measured signal when electrochemical detectors working on

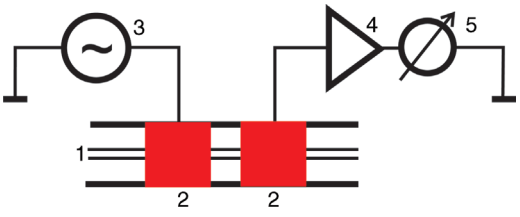

Figure 11.6 Basic scheme of a capacitively coupled contactless conductivity detector for CE–C^4D. Separation capillary (1); conductivity electrodes (2); alternating signal generator (3); detector electronics – alternating current meter, rectifier, and amplifier (4); and signal recorder (5).

different principles are used. Simultaneously, the effect of the separation electric field on the measured signal is eliminated.

Contactless detection is facilitated by bringing an alternating signal with a frequency on the order of tens of kHz to units of MHz and amplitude U on the order of tens of volts to the conductivity electrodes. The signal passes through the walls of the capillary and the solution in the capillary between the electrodes and is recorded by the other electrode (Figure 11.6). The analytical signal is the passing alternating current, I, which depends on the impedance of the conductivity cell, Z, determined both by the parameters of the detection cell, which can be considered constant, and the resistance of the solution in the capillary, which changes on passage of the analyte zones.

$$I \approx U/Z. \tag{11.6}$$

The direction of the response of the conductivity detector is controlled by KRF similar to indirect UV detection. In the analyte zone, part of the BGE coions is replaced by analyte ions; this effect is quantified by TR (see above). If the effective mobility of the analyte u_A is greater than the effective mobility of the coion u_C, the analyte zone exhibits greater conductivity than the surrounding BGE and appears as a positive peak in the conductivity detector. When $u_A < u_C$, it appears as a negative peak or dip. The composition of the BGE must be optimized from the viewpoint of conductivity detection so that the difference between u_A and u_C is as great as possible. On the other hand, this is disadvantageous from the viewpoint of broadening of peaks through electrodispersion. (The described simplified theory of conductivity detection is fully valid for completely dissociated electrolytes; exceptions exist for weak electrolytes.)

The C^4D method is characterized by lower sensitivity than, for example, LIF or MS detection, but does not require derivatization of the monitored analytes and is instrumentally very simple. It can be readily used with buffers with low mobility coions, contributing to more sensitive detection. In addition, because of the low conductivity of the separation medium, lower current passes through the capillary and this generates less Joule heat; thus, one of the phenomena reducing the separation efficiency is suppressed.

C^4D has recently become a standard detection technique especially for the detection of small inorganic high-mobility ions [22], for which it attains 10 times greater sensitivity compared to indirect UV detection; in addition, it is extensively used for the detection of amino acids, saccharides, and other substances that do not absorb UV radiation. Its rapid development over the past 15 years can truly be called a renaissance of impedance detection methods [23]. The theoretical foundations of the method, which have been mentioned in a great many publications, were summarized and critically discussed in Refs. [24,25]. Its high practical potential is regularly documented in published works, for example, [26–28] dealing with instrumental and application aspects of contactless detection. C^4D is commercially available (www.eDAQ.com; www.istech.at) and it can be anticipated that it will soon be supplied by the manufacturers of electrophoresis apparatuses as a standard detection method in parallel with a UV detector.

11.3.2.2 Detection Cell

Conductivity detectors are most frequently tubular or semitubular with a length of units of millimeter and distance apart of tenths to units of millimeter. The detection occurs approximately in the capillary volume between the electrodes. The capillary passes through tubular electrodes, while it is closely fitted into semitubular electrodes. The electrodes can be an integral part of the capillary, that is, they can be formed, for example, by a conductive coating directly on its surface; this arrangement is preferable from the viewpoint of the noise of the monitored signal, but does not permit replacement of a damaged capillary. When tubular electrodes are used, it is necessary to fix the capillary so as to prevent it from moving inside the electrode, to suppress excessive noise. The inner diameter of tubular electrodes must be somewhat larger than the outer diameter of the capillary to facilitate replacement where required; a change in the distance of the capillary from the electrode walls is a source of undesirable noise. The entire detection cell must be placed in a Faraday cage. In some cases, a shielding foil placed in the space between the electrodes is recommended to reduce undesirable transmission of an alternating signal between the electrodes along the surface of the capillary. The parasitic signal transmission may be decreased but not eliminated completely [25]; moreover, it makes the cell more complicated.

As mentioned above, a high-frequency alternating (usually sine-wave) current is brought to one of the electrodes. Detailed optimization procedures on how to choose a suitable frequency and amplitude of the alternating signal to attain the required signal/noise ratio are described in the literature. However, in practice, a frequency on the order of hundreds of kHz and amplitude of about 20 V pp (peak-to-peak) is used most often. The alternating current flowing between the electrodes following rectification and filtering is recorded.

A contactless conductivity cell has the great advantage that it can be moved along the whole length of the separation capillary and that the analyte zones can

be detected without it being necessary to remove the polyimide coating from the capillary. The ability to place C^4D at any site along the capillary is utilized for the simultaneous determination of cations and anions. Samples in which cations and anions are to be determined are injected into the opposite ends of the capillary and the C^4D is placed close to the central part of the capillary to ensure optimal detection of both kinds of ions [29,30]; a pair of C^4D placed at opposite ends of the capillary were used for the same purpose [31]. An array of sixteen C^4D placed at regular distances along the capillary permits detailed monitoring of the separation process [32].

11.3.3 Electrochemiluminescence Detection

11.3.3.1 Principles of the Method and General Characteristics

Electrochemiluminescence (ECL) detection is based on measuring the intensity of radiation formed upon the reaction of an electrochemically generated product with the analyte leaving the separation capillary; the product is generated amperometrically at an electrode fixed immediately next to the end of the capillary. At present, this product is almost always generated from tris (2,2′-bipyridyl)-ruthenium (II), $[Ru(bpy)_3]^{2+}$. This substance is characterized by good stability in aqueous and nonaqueous solutions and electrochemical generation of the active product, $[Ru(bpy)_3]^{3+}$, and is kinetically not complicated. Direct ECL is used primarily for the detection of amines and their derivatives, where its sensitivity increases substantially in the series primary < secondary < tertiary amines. The detection mechanism can be depicted by the simplified scheme:

Oxidation at the electrode	$[Ru(bpy)_3]^{2+} - e^- \rightarrow [Ru(bpy)_3]^{3+}$
Reaction in solution	$[Ru(bpy)_3]^{3+} + \text{amine group} \rightarrow [Ru(bpy)_3]^{2+*}$
Luminescence in solution	$[Ru(bpy)_3]^{2+*} \rightarrow [Ru(bpy)_3]^{2+} + hf$,

where $[Ru(bpy)_3]^{2+*}$ is the product in the excited state that emits radiation with a wavelength of 610 nm when it returns to the ground state.

Similar to AD, the effect of the separation electric field on the ECL signal has been discussed. This effect is disputable; in some works, the use of off-capillary detection with a decoupler or the use of a capillary with i.d. < 25 μm and low-conductivity separation buffer is recommended, in others only the effect of the optimum generation potential of the electrode and not the effect of separation electric field on the ECL signal is discussed.

A great many substances, for example, phenolic compounds, extinguish luminescence and this can be used for their indirect ECL detection. In this case, electrochemiluminescence is generated at the exit end of the capillary, for example, in the $[Ru(bpy)_3]^{2+}$/tripropylamine system. The analytes leaving the capillary are manifested in a decrease in the intensity of the detected radiation. Detailed

information on the methodology and applications of ECL can be found in a number of reviews, for example, [33–36].

11.3.3.2 Detection Cell

ECL detection combines electrochemical and optical principles and thus the detection cells are more complicated than C^4D cells. Thus, the efficiency of the ECL detection depends on a number of factors, which must be taken into consideration when designing detection cells and that must be optimized for solving specific analytical tasks. These are primarily the distance between the end of the capillary and the generation electrode (tens to hundreds of μm), the size and material of the generation electrodes (similar to AD, bare or chemically modified Pt, Au, various forms of carbon, including CNT, BDD, and indium-tin oxide (ITO)), the generation potential (approxmately 1–1.5 V versus Ag/AgCl, optimized by cyclic voltammetry), the concentration of $[Ru(bpy)_3]^{2+}$ (on the order of 10^{-4} mol/l), and the composition of the BGE. Thus, a compromise must frequently be made – for example, the BGE should be satisfactory for both the separation and the detection process.

The sensitivity of the detection is also greatly affected by the position of the PMT recording the radiation in relation to the site of the radiation generation. The radiation generated at the electrode is scattered in all directions to the surroundings. A frequently used arrangement of the detection cell (Figure 11.7a) is unsuitable from this viewpoint because the radiation is recorded in only one direction. An arrangement with a ring-shaped electrode is effective [37] (Figure 11.7b), where almost 100% of the generated radiation is recorded. A transparent conductive material, for example, ITO [38], can be used as the generating electrode. Problems associated with exact adjustment of the position of the generation electrode in relation to the end of the capillary are avoided in the wall-jet arrangement (Figure 11.7c), similar to when ring electrodes are used.

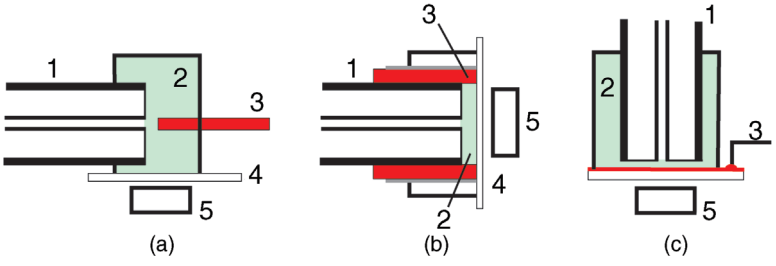

Figure 11.7 Examples of geometric arrangements of detection cells for CE-ECL detection. (a) Standard arrangement with a microelectrode close to the end of the capillary, (b) cell with ring electrode, (c) cell with electrode composed of ITO on a transparent base. Capillary (1), detection cell (2), generation electrode (3), optically transparent window (4), and PMT (5). The auxiliary and reference electrodes are not depicted; the detection cells are placed in a dark box.

The manner of adding the reaction agent $[Ru(bpy)_3]^{2+}$ to the site of generation of electrochemiluminescence is another important parameter in designing ECL detection cells. In principle, three arrangements can be used:

1) *Precapillary reagent addition*: The reagent is added directly to the BGE. This has the advantage of simple cell design, but unspecific adsorption of the reagent on the walls of the capillary affects EOF and the separation efficiency. The separation and detection processes cannot be optimized independently.
2) *Postcapillary reagent addition*: The reagent is added to the detection cell at the end of the capillary. This is the commonest arrangement for ECL detection. In the static arrangement, it is necessary to frequently change the solution with the reagent in the detection cell because it is diluted by the eluent from the capillary. In the dynamic arrangement, the reagent solution flows continuously through the detection cell, either under the effect of gravitation or the flow rate is controlled by a mechanical pump; the flow rate is on the order of μl per min. In addition to optimization of the experimental conditions, it is necessary to also consider the consumption of expensive reagent.
3) Immobilization of the reagent in a polymer film fixed to the surface of the platinum electrode – called *solid-state ECL detection*. In this case, the detection cell is simple, a change in the composition of the reaction solution by the eluent is eliminated and the reagent consumption is minimized.

11.4 Combinations of Detection Techniques

In the analysis of more complicated samples, standard CE detectors do not permit detection of all the analytes of interest in the sample. A typical example consists of samples containing small inorganic ions that cannot be detected directly using an optical detector in a mixture with organic ions containing a chromophore that can be readily detected optically. One of the ways in which analytes of different character can be detected and determined in a single separation in a single analysis is to use detection by a combination of two (or more) detectors based on different physical–chemical principles. A survey and details of combined detection methods in CE can be found, for example, in the review [21]; this work also discusses dual AD–AD detection, mentioned above. In dual detection (and, in general, in multidetection), it is desirable if

1) the detection systems enable detection at one site in the separation capillary; however, for spatial reasons, they are generally connected in series, so that the record from the individual detectors is shifted in time and corresponds to different degrees of separation of the substances.

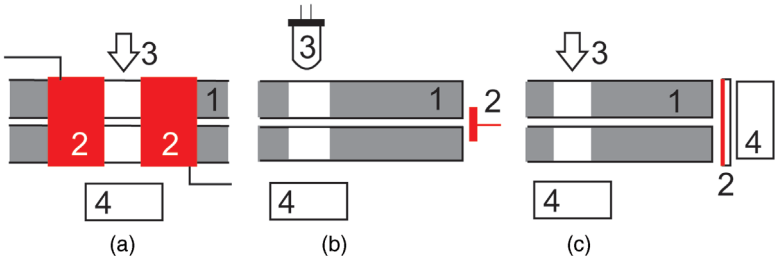

Figure 11.8 Basic schemes of some dual detectors (a) Combination of C^4D with optical detection (UV, LIF, and LEDIF), (b) combination of optical detection with amperometric detection, and (c) combination of optical detection with electrochemiluminescence (ECL is generated at an ITO electrode on transparent material) or chemiluminescence (a generation electrode is not required). Capillary (1), electrodes (2), radiation source (3), and radiation detector (4).

2) the detection is performed on-capillary, that is, to preserve the efficiency of the separation process.

Both these requirements are met by C^4D in combination with optical detection (Figure 11.8a). Radiation can be brought to the detection window between the conductivity electrodes, for example, by an optical fiber, with absorption (C^4D-UV) or fluorescence (C^4D-LIF) detection. The transmitted radiation in absorption or the emitted radiation in fluorescence detection can be brought to the detection element again by an optical fiber, or a large-area photodiode or PMT is directly applied close to the detection window. In fluorescence detection, the emitted radiation can be recorded by an optical fiber applied to the capillary in the direction perpendicular to the excitation radiation. This advantageous arrangement cannot be used in commercial electrophoretic apparatuses with a capillary located in a cassette and thus the conductivity and optical detection cells are connected in series at a distance of approxmately 10 cm in these arrangements. A combination of C^4D with other detection methods is used most frequently in CE.

LED radiation sources are frequently used to advantage in dual detectors containing an optical detection cell in laboratory CE systems. They have also been used in on-capillary detectors combining three detection principles, C^4D, UV, and LEDIF [39], at a single place on the capillary.

In some dual detectors, both detection cells cannot be in the on-capillary arrangement for fundamental reasons. These are detectors combining optical detection (UV, LIF, and LEDIF) with amperometric detection (Figure 11.8b), electrochemiluminescence detection (Figure 11.8c), or chemiluminescence. For detectors combining amperometric and electrochemiluminescence detection, the scheme is similar to that in Figure 11.7a; or, dual amperometric detectors (Figure 11.5c) can be used only in the off-capillary arrangement.

Dual detection has great practical importance. In addition to the basic function, that is, the ability to detect separated substances with different character,

substances can be determined in samples where they are not completely separated or even migrate in a single zone, provided that they don't both yield signals in both detectors. In addition, signals from detectors working on different principles greatly assist in identification of the migrating substances.

In multidetection, the BGE composition should conform to both the separation process and the detection methods. Except one's own experience, the paper [8] can help in BGE optimization at resolving a particular analytical task. At present, practically all the dual detection systems used to date were made in the laboratory, generally designed to solve a certain specific analytical problem.

11.5 Conclusions

This chapter summarizes detectors commonly used for CE, many of which are commercially available (UV-Vis absorbance, diode array detection, fluorescence detection, laser-induced fluorescence, and contactless conductivity detection). In addition, a number of less common detection principles can be used, such as refractive index detection, nuclear magnetic resonance, Raman spectroscopy, infrared spectroscopy, chemiluminescence, radioisotope detection, and others. Detectors based on these principles have limited use and are employed only for special analyses.

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