In gradient elution, detection requires quantitative measurement of the sample components in an eluent whose composition and, hence, physical properties alter in the course of the analysis. Even solvent combinations purposefully selected with respect to a certain property, e.g. “iso-refractive” solvents, cannot ensure proper measurements of small solute concentrations by detectors which monitor only a bulk property of the mobile phase plus the solute.

The detection problem in gradient elution can be solved by either using a selective detector sensitive to a property of only the solute (see Sect. 7.1) or stripping off the eluent with subsequent measurement of non-volatile residues (see Sect. 7.2).

The current status and further prospects of HPLC detectors have been dealt with in a recent review article [1], which compares the limits of detection for twelve different kinds of detectors, and in monographs [2, 3].

7.1 Selective Detectors

Selective devices are (1) UV/visible photometers and spectrophotometers, (2) infrared photometers, (3) amperometric detectors, (4) reaction detectors, as well as radioactivity detectors, fluorometers, optical rotation detectors, circular dichroism detectors, and laser light scattering detectors.

7.1.1 UV/Visible Photometers and Spectrophotometers

Photometers for UV and visible light are most common detectors in HPLC. The cells typically have an optical path length of 10 mm and a volume of about 8 μl. The flow path is Z-shaped in order to minimize stagnant regions in the cell. Quartz windows are pressed against the core of the cell.

The signal from cylindrical cells may be influenced by changes in refractive index which form “liquid lenses” deflecting light towards the cell walls. They reduce the energy reaching the light sensor and produce pseudopeaks which do not belong to UV absorption [4]. The effect is especially pronounced at sudden changes in gradient steepness. Modern absorption detectors have tapered flow cells with diverging walls [5] where light is prevented from hitting the walls even if the refractive index of the mobile phase is changing.

The windows of a flow cell may sometimes be not strictly parallel. Wedge angles varying from 0.5 to 2° were found in a painstaking investigation [6]. A wedged cell behaves like a prism and, thus, also causes light deviation on
changing refractive index. The transmitted beam wanders around on the surface of the photodiode where local response inhomogeneities can generate small changes in signal size due to changes of the mobile phase or to incomplete mixing in the detector cell.

### 7.1.2 Infrared Photometers

Detectors of this kind permit stable operation with a heated cell up to 150 °C. They are suited for high-temperature separations of synthetic polymers and are used, e.g. in SEC of polyolefins or temperature-rising elution fractionation (see Sect. 9.14).

In gradient HPLC, IR detectors can be used when eluent mixtures are available which have suitable “windows”, i.e. no absorption in a wavenumber range where the copolymer can be detected. Simple copolymers of known structure can be analysed this way if suitable eluents can be found.

For instance, an IR detector has been used for selectively monitoring at 1730 cm⁻¹ the MMA content of S/MMA copolymers in gradient elution with hexane/chloroform by measuring the carbonyl absorption. The styrene content could be monitored by UV absorption at 254 nm, see Fig. 9.4 [7].

In general, the gradient components may disturb the application of IR detection. In that case a deposition technique can be applied which also enables the whole IR spectra of the eluite to be measured. In this technique, the column eluate is sprayed onto a substrate, which is slowly moved beneath the effluent spray. The solvents are vaporized by heating. Nonvolatile sample components remain spatially separated on the substrate and can be analysed by Fourier transform IR. The technique has been used so far with supercritical fluid chromatography [8–11].

### 7.1.3 Amperometric Detectors

A device of that kind requires conductivity of the eluent. This problem can be circumvented by postcolumn addition of a solvent with a high dielectric constant plus supporting electrolyte.

Nitrocellulose could be selectively detected by electrochemical reduction at a pendent mercury drop electrode held at zero potential vs Ag/AgCl electrode. An appropriate level of insulation at a minimal contact with oxygen-permeable PTFE allowed for a background current not larger than $2 \times 10^{-9}$ A. The device enabled nanogram amounts of nitrocellulose to be characterized [12].

### 7.1.4 Reaction Detectors

Chemical derivatization by post-column reactions is another approach to selective detection. For a review, see References [13,14] and the literature cited there.

With synthetic polymers, post-column reaction-detection has been performed with the help of an ozone reaction detector which monitores double bonds. The device has been employed in SEC investigations of polybutadiene and EPDM rubber [15] as well as of polyisobutylene [16].