Detectors: Refractive Index Detectors

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Introduction

The refractive index detector is a bulk property detector. A bulk property detector responds to some physical property of the total column eluent and not some specific property of the solute. Bulk property detectors have an inherently limited sensitivity, irrespective of the instrumental technique used. Consider a hypothetical bulk property detector that is to monitor the density of the column eluent. Assume it is required to detect the concentration of a dense material, such as carbon tetrachloride (specific gravity 1.595), at a level of 1 μg mL^{-1} in *n*-heptane (specific gravity 0.684).

This situation will be particularly favourable for this hypothetical detector, as the solute to be sensed exhibits a large difference in density from that of the solvent. Let the change in density resulting from the presence of the solute at a concentration of 10^{-6} g mL^{-1} be Δd. It follows that, to a first approximation:

$$\Delta d = \frac{X_S(d_1 - d_2)}{d_1} \qquad [1]$$

where d_1 is the density of the solute, carbon tetrachloride, d_2 is the density of the mobile phase, *n*-heptane, and X_S is the concentration of the solute to be detected.

Thus, for the example given:

$$\Delta d = \frac{(1.595 - 0.684) \times 10^{-6}}{1.59} = 5.71 \times 10^{-7} \qquad [2]$$

Now the coefficient of cubical expansion of *n*-heptane is approximately 1.6×10^{-3} per °C. Thus, the temperature, ΔT, that would produce a change in density equivalent to the presence of carbon tetrachloride at a concentration of 10^{-6} g mL^{-1} can be calculated.

It follows that:

$$\Delta T = \frac{5.71 \times 10^{-7}}{1.6 \times 10^{-3}} °C$$

$$= 3.6 \times 10^{-4} °C \qquad [3]$$

Assuming that a concentration of one part per million carbon tetrachloride is just detectable (it provides a signal-to-noise ratio of 2), then the temperature fluctuations must be maintained below 1.8×10^{-4}°C to achieve this sensitivity. In practice, such temperature stability would be extremely difficult to maintain and thus the temperature control can severely limit the sensitivity obtainable from such a detector. Even the heat of adsorption and desorption of the solute to and from the stationary phase can easily result in temperature changes of this order of magnitude. The density of the contents of the cell will also change with pressure and, if there is a significant pressure drop across the cell, with flow rate. These restrictions apply to all bulk property detectors and so all bulk property detectors will have a limited sensitivity determined by the stability of the ambient conditions. This limit of detection is probably around 10^{-6} g mL^{-1}.

The refractive index detector was one of the first online detectors to be developed and was described by Tiselius and Claesson in 1942. It was also one of the first online liquid chromatography (LC) detectors to be made commercially for general use. The refractive index detector is probably the least sensitive of the commonly used LC detectors. Its major disadvantage (as already discussed) is its sensitivity to changes in ambient conditions, such as temperature, pressure and flow rate. Another handicap is that it cannot be used for gradient elution, due to the continuous change in mobile-phase refractive index that results from the change in solvent composition. Nevertheless, as the refractive index detector has a universal response, it can be extremely useful for detecting those compounds that are nonionic, do not absorb in the UV and do not fluoresce (e.g. aliphatic alcohols, fatty acids, ethers, etc.).

When a monochromatic ray of light passes from one isotropic medium, A, to another, B, it changes its velocity and direction. The change in direction is called the refraction, and the relationship between the angle of incidence and the angle of refraction is given by Snell's law:

$$n'_B = \frac{n_B}{n_A} = \frac{\sin(i)}{\sin(r)} \qquad [4]$$

where i is the angle of incident light in medium A, r is the angle of refractive light in medium B, n_A is the refractive index of medium A, n_B is the refractive index of medium B and n'_B is the refractive index of medium B relative to that of medium A.

Refractive index is a dimensionless constant that normally decreases with increasing temperature; values given in the literature are usually quoted at 20°C or 25°C, the actual measurement taken as the mean value for the two sodium lines. If a cell takes the form of a hollow prism through which the mobile phase flows, a ray of light passing through the prism will be deviated from its original path. If the light is focused on to a photocell the output will change as the refractive index of the mobile phase in the cell changes. This method of monitoring refractive index is called the angle of deviation method and has been used by a number of manufacturers in their detector design.

The modern refractive index detector is the result of considerable research which has been extended by the research and development laboratories of many instrument companies. A diagram of a simple refractive index detector based on the angle of deviation method measurement is shown in **Figure 1**. A beam of light from an incandescent lamp passes through an optical mask that confines the beam to the region of the cell. The lens collimates the light beam, which passes through both the sample and reference cells to a plane mirror. The mirror reflects the beam back through the sample and reference cells to a lens, which focuses it on to a photocell.

The location of the beam, rather than its intensity, is determined by the angular deflection of the beam caused by the difference in refractive index between the contents of the two cells. As the beam changes its position of focus on the photoelectric cell, the output changes and the resulting difference signal is electronically modified to provide a signal proportional to the concentration of solute in the sample cell.

An alternative method of refractive index measurement, the Fresnel method, has also been used in the design of commercially fabricated detectors. The two different systems provide comparable performance with respect to sensitivity and linearity, and mostly differ in the manufacturing techniques used to construct the instruments. The relationship between the reflectance from an interface between two transparent media, and their respective refractive indices, is given by Fresnel's equation:

$$R = \frac{1}{2}\left[\frac{\sin^2(i-r)}{\sin^2(i+r)} + \frac{\tan^2(i-r)}{\tan^2(i+r)}\right] \qquad [5]$$

where R is the ratio of the intensity of the reflected light to that of the incident light and the other symbols have the meanings previously assigned to them. Now:

$$\frac{\sin(i)}{\sin(r)} = \frac{n_1}{n_2} \qquad [6]$$

where n_1 is the refractive index of medium 1 and n_2 is the refractive index of medium 2.

Consequently, if medium 2 represents the liquid eluted from the column, then any change in n_2 will result in a change in R and thus the measurement of R could determine changes in n_2 resulting from the presence of a solute. Conlon utilized this principle to develop a practical refractive index detector. His device, now obsolete, illustrates the principle of the Fresnel method very simply (**Figure 2**).

The sensing element consists of a rod prism sealed into a tube through which the solvent flows. The rod prism is made from a glass rod 6.8 mm in diameter

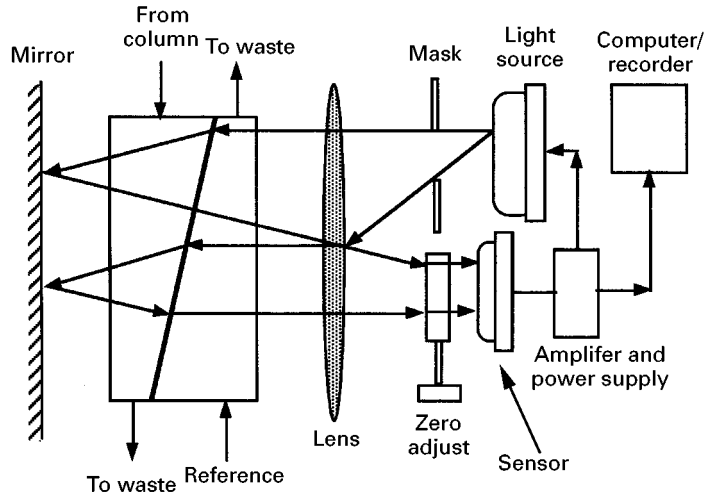

Figure 1 The refractive index detector based on the angle of deviation method of measurement. (Courtesy of Waters Chromatography.)

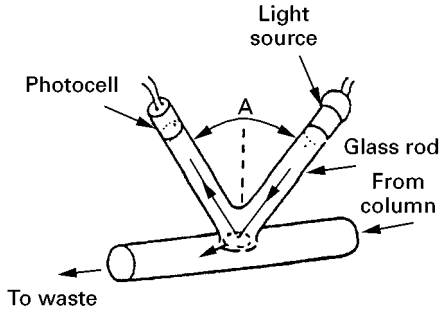

Figure 2 Early detector based on the Fresnel method of refractive index measurement.

and 10 cm long, bent to the correct optical angle (just a little less than the critical angle) and an optical flat is ground on the apex of the bend, as shown in Figure 2. The optical flat is then sealed into the window of a suitable tube that acts as a flow-through cell. The photocell is arranged to be one arm of a Wheatstone bridge and a reference photocell (not shown) monitoring light direct from the cell is situated in another arm of the bridge.

This detector was never manufactured as it had too large a cell volume and limited sensitivity. However, it was one of the first refractive index detectors to work on the Fresnel principle. A commercial refractive index detector that works on this principle is shown in **Figure 3**.

Light from a tungsten lamp is directed, through an infrared filter to prevent heating the cell, to a magnifying assembly that also splits the beam into two. The two beams are focused through the sample and reference cells respectively. Light refracted from the mobile phase/prism surface passes through the prism assembly and is then focused on two photocells. The prism assembly also reflects light to a user port where the surface of the prism can be observed. The output from the two photocells is electronically processed and either passed to a potentiometric recorder or a computer data acquisition system.

The range of refractive index covered by the instrument for a given prism is limited and consequently three different prisms are made available to cover the refractive index ranges of 1.35–1.4, 1.41–1.44 and 1.40–1.55 respectively. An example of the separation of a series of polystyrene standards monitored by the detector is shown in **Figure 4**. The separation was carried out by size exclusion on a column packed with 5 μm particles operated at a flow rate of 0.8 mL min^{-1}.

The Christiansen Effect Detector

This method of measuring refractive index arose from the work of Christiansen on crystal filters. If a cell is packed with particulate material having the same refractive index as the mobile phase passing through it, light will be transmitted through the cell with little or no refraction or scattering. If, however, the refractive index of the mobile phase changes, there will be a refractive index difference between the mobile phase and that of the packing. This difference will result in light being refracted away from the incident beam and thus reduce the intensity of the transmitted light. If the transmitted light is focused on to a photocell, and the refractive index of the packing and mobile phase initially matched, then any change in refractive index resulting from the elution of a solute peak will cause light scattering. This scattering will reduce the intensity of the light falling on the sample photocell and thus provide a differential output relative to that of the reference cell.

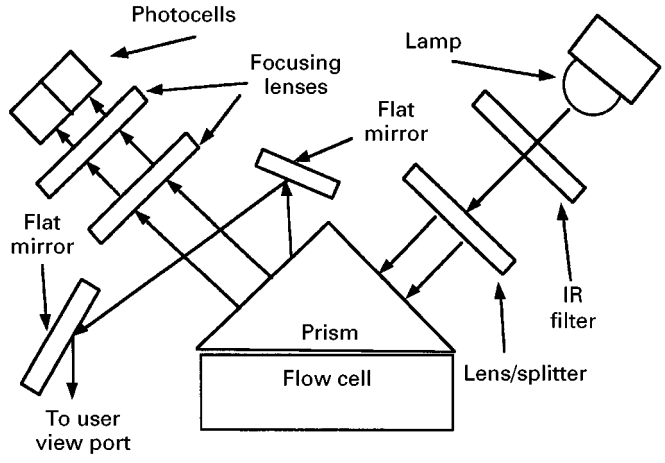

Figure 3 Diagram of the optical system of a refractive index detector operating on the Fresnel principle. (Courtesy of Perkin Elmer.)

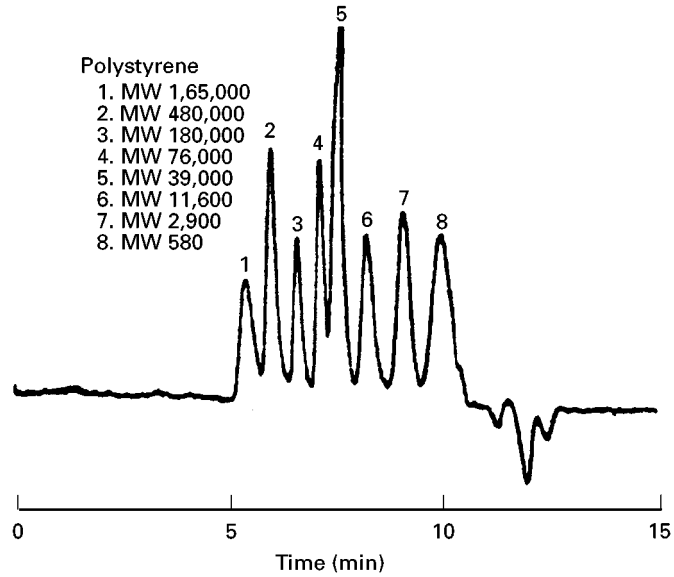

Figure 4 Separation of some polystyrene standards using a refractive index detector operating on the Fresnel principle. (Courtesy of Perkin Elmer.)

As the optical dispersions of the media are likely to differ, the refractive indices of the packing and the solvent will only match at one particular wavelength. Consequently, the fully transmitted light will be largely monochromatic. Light of other wavelengths will be proportionally dispersed depending on their difference from the wavelength at which the two media have the same optical dispersion. It follows that a change in refractive index of the mobile phase will change both the intensity of the transmitted light and its wavelength content.

A Christiansen detector is shown in **Figure 5**. The optical module contains a prefocused lamp, the voltage of which is adjustable to allow operation at low energy when the maximum sensitivity is not required. The condensing lens, aperture, achromat and beam-splitting prisms are mounted in a single tube to prevent contamination from dust and permit easy optical alignment. The system has two identical and interchangeable cells. The disadvantage of this detector is that the cells must be changed when alternative mobile phases are used in order to have a packing with

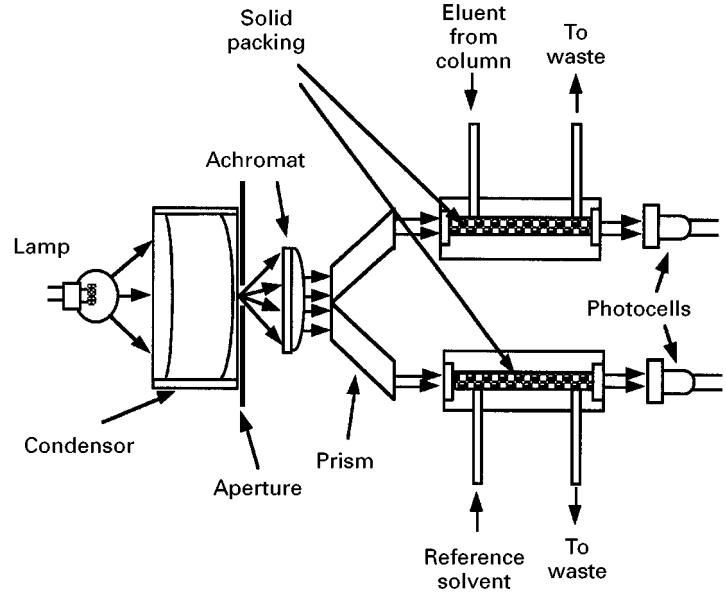

Figure 5 The Christiansen effect detector.

the appropriate refractive index. Close matching of the refractive indices of the cell packing and the mobile phase can be achieved by using mixed solvents.

Solvent mixing can usually be achieved without significantly affecting the chromatography distribution system, e.g. a small proportion of *n*-heptane in a mixture can be replaced with either *n*-hexane or *n*-octane either to increase or decrease the refractive index. The limitations inherent in this type of refractive index measurement, taken in combination with general disadvantages of the refractive index detector *per se*, have prevented this detector becoming very popular. This device has been claimed to have a sensitivity of 1×10^{-6} refractive index (RI) units. This would be equivalent to a sensitivity of 9×10^{-6} g mL^{-1} of benzene (RI = 1.501) eluted in *n*-heptane (RI = 1.388). The cell was designed to have a minimum volume of 8 μL, which is slightly large for modern sensors, but small enough to work well with normal 4.6 mm i.d. columns. Different cells packed with appropriate materials were necessary to cover the refractive index range of 1.31–1.60.

The Interferometer Detector

The interferometer detector was first developed by Bakken and Stenberg in 1971. The detector responds to the change in the effective path length of a beam of light passing through a cell, when the refractive index of its contents changes, due to the presence of an eluted solute. If the light transmitted through the cell is focused on a photocell coincident with a reference beam of light from the same source, interference fringes will be produced. These fringes will change, as the pathlength of one light beam changes with reference to the other. Consequently, as the concentration of solute increases in the sensor cell, a series of electrical pulses will be generated as each fringe passes the photocell.

The effective optical path length (d) depends on the change in refractive index (Δn), and the path length (l) of the sensor cell:

$$d = \Delta n l \qquad [7]$$

It is possible to calculate the number of fringes (N: sensitivity) which move past a given point (or the number of cyclic changes of the central portion of the fringe pattern) in relation to the change in refractive index by the equation:

$$N = \frac{2\Delta n l}{\lambda} \qquad [8]$$

where λ is the wavelength of the light employed.

As N increases for a given refractive index change, Δn, so will the detector sensitivity. Therefore l should be made as large as possible commensurate with the chromatographic properties of the system. The simple optical system originally employed by Bakken and Stenberg is shown in **Figure 6**. Light from a source strikes a half-silvered mirror and is divided into two paths. Part of the beam is reflected by a plane mirror back along the same path and on to a photocell. The other part of the beam passes through the sensor cell to a plane mirror, where it is reflected back again through the sensor cell to the half-silvered mirror that reflects it on to the photocell where interference takes place with the other half of the light beam.

The number of fringes that pass the sensor will be directly proportional to the total change in refractive index, which will be proportional to the total amount of solute present. Although it establishes the technical viability of the system, the apparatus has limited use, but it has been developed into a practical instrument and an example is that developed by Wyatt Technology. The optical system of the Optilab interference detector is shown in **Figure 7**.

Light from an appropriate source is linearly polarized at -45° to the horizontal plane. Horizontal and vertical polarized light beams are produced and, on passing through the Wollaston prism, one passes through the sample cell and the other through the reference cell. The beam passing through the sample cell is horizontally polarized and that through the reference cell is vertically polarized. After passing through the cells, the beams are focused on a second Wollaston prism and then through a quarter-wave plate which has its fast axis set -45° to the horizontal plane.

A beam that is linearly polarized in the fast-axis plane will, after passing through the plate, lead another linearly polarized, but orthogonal, beam by a quarter of a wavelength. The phase difference results in a circularly polarized beam. It can be assumed that each of the beams focused on the Wollaston prism consists of two such perpendicular beams which, after the quarter-wave plate, result in two circularly polarized beams of opposite rotation. These beams will interfere with each other to yield the original linearly polarized beam. A second polarizer is placed at an angle $(90 - \beta)$ to the first (for the significance of β, see below), allowing about 35% of the signal to reach the photocell. A filter-transmitting light at 546 nm precedes the photocell.

If the sample cell contains a higher concentration of solute than the reference cell, in general the refractive index will be higher and the interfering beams will be out of phase. The refractive index difference (Δn) and

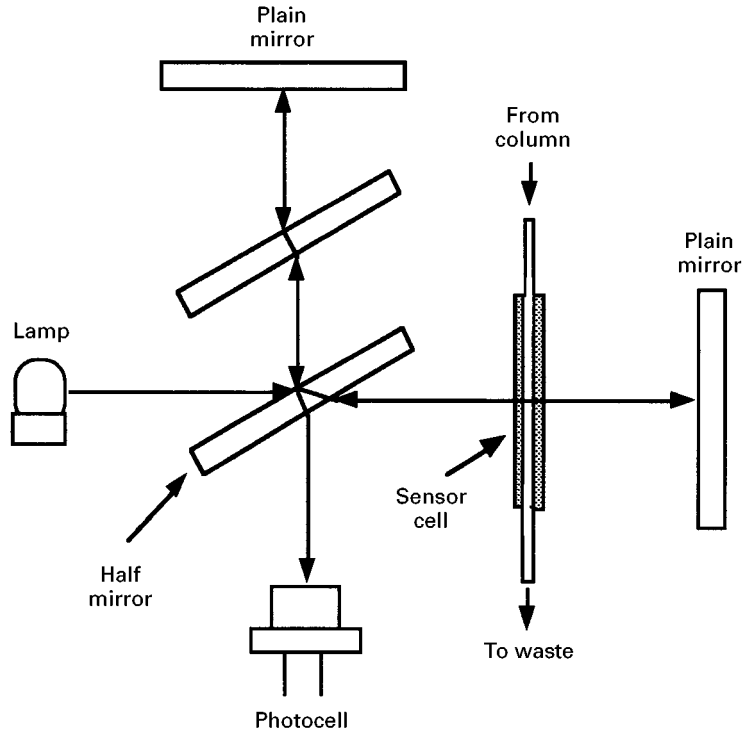

Figure 6 The original optical system used by Bakken and Stenberg in their interferometer detector.

the phase difference (Δp) are related by:

$$\Delta p = \frac{2\pi L \Delta n}{\lambda} \qquad [9]$$

where L is the length of the cell and λ is the wavelength of the light.

The circularly polarized beams will interfere to yield a linearly polarized beam which is rotated $\Delta p/2$ rad, and the amplitude of the light striking the photocell (A_p) will be given by:

$$A_p = A_0 \cos\left(90 - \beta - \frac{\Delta p}{2}\right) = A_0 \cos\left(\beta - \frac{\Delta p}{2}\right) \qquad [10]$$

The smallest cell (1.4 μL: a cell volume that would be suitable for use with microbore columns) is re-

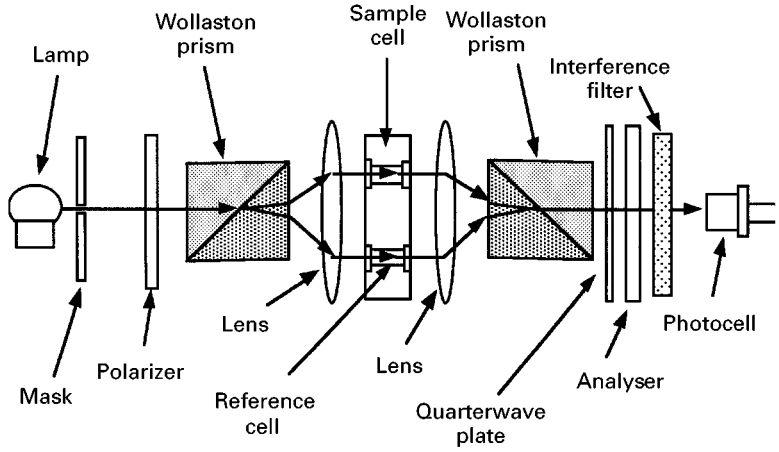

Figure 7 The Optilab interference refractometer detector.

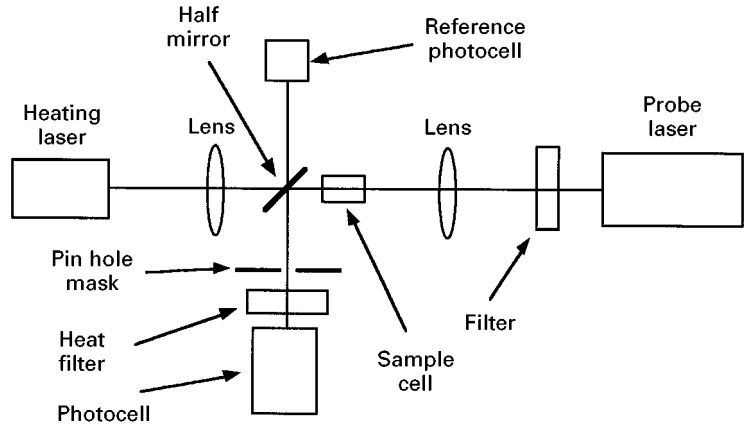

Figure 8 The layout of a thermal lens detector.

ported to give a sensitivity of about 2×10^{-7} RI units at a signal-to-noise ratio of 2. Consequently, for benzene (RI = 1.501) sensed as a solute in *n*-heptane (RI = 1.388), this sensitivity represents a minimum detectable concentration of 5.6×10^{-5} g mL^{-1}. The alternative 7 μL cell would decrease the minimum detectable concentration to about 1×10^{-6} g mL^{-1}, similar to that obtained for other refractive index detectors. However, this cell volume is slightly large for modern high efficiency columns.

The Thermal Lens Detector

There are a number of detectors that have been developed that are not classed as refractive index detectors, but their response is either based directly on refractive index measurement, or a function of some physical property of the mobile-phase system that is related to the refractive index. One such example of these is the thermal lens detector.

When a laser is focused on to an absorbing substance, the refractive index may be changed and modify the medium in such a way that it behaves as a lens. This phenomenon was first reported by Gorden *et al.* in 1964 and subsequently the effect has been examined by a number of workers. The formation of the thermal lens is caused by the absorption of laser light which may be extremely weak. The excited-state molecules subsequently decay to the ground state, with a resulting localized temperature increase in the sample. As the refractive index of the medium depends on the temperature, the spatial variation of the refractive index in the medium produces the phenomenon which appears to be equivalent to the formation of a lens within the medium. The temperature coefficient of refractive index is, for most liquids, negative; consequently, the insertion of a liquid in the laser beam produces a concave lens that results in beam divergence. The thermal lens effect has been used by Buffet and Momis to develop a small volume detector. Their system is shown in **Figure 8**.

The device consists of a heating laser, from which light is passed directly through the sample via two lenses and a half mirror. Another laser, the probe laser, passes light in the opposite direction, through one lens, then through the sample to the half mirror where the light is reflected on to a photocell. Between the mirror and the photocell is a filter to remove the heating laser light and a small pinhole aperture. When an absorbing solute arrives in the cell, a thermal lens is produced which causes the probe light to diverge, and consequently the intensity of the light passing through the pinhole and on to the photocell is reduced. The cell can be made a few microlitres in volume and would thus be suitable for use with microbore columns. A sensitivity of 10^{-6} AU (the expected limiting sensitivity of a bulk property detector) was claimed for the detector and a linear dynamic range of about three orders of magnitude.

The use of two lasers adds significantly to the cost of the device. Basically, the thermal lens detector is a special form of the refractive index detector and as a consequence can be considered as a type of universal detector. However, it cannot be used with gradient elution or flow programming and its sensitivity is no better than other refractive index detectors.

Conclusion

Despite the refractive index detector being the oldest and least sensitive of all the LC detectors, its use survives, and will continue to survive, due to its universal response. All other LC detectors that are in use have sensors that only respond to certain types of solutes and therefore are restricted to specific sample types. At any time a new type of catholic detecting system might be devised, but

after 30 years of detector development, only the evaporative light-scattering detector has offered any viable alternative. As a consequence, the future of the refractive index detector for specific applications still appears assured. In most applications an isocratic development procedure can be found that will provide satisfactory resolution of those solutes of interest, and so the problem of gradient elution can be circumvented. In addition, by using columns of 4.6 mm i.d. or more, the limited sensitivity of the refractive index detector can also be accommodated.

Further Reading

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Detectors: UV/Visible Detection

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The UV absorption detector, introduced in the early 1960s, was the first high sensitivity liquid chromatography detector to be developed. UV absorption detectors are considered to be those detectors that can sense substances that absorb light over the wavelength range 180–350 nm. Many compounds absorb light in this range, including those substances having one or more double bonds (π electrons) and those that have unshared (unbonded) electrons, e.g. all olefins, all aromatics and compounds, for example, containing $>C=O$, $>C=S$ and $-N=N-$ groups. As a result, providing the wavelength of the incident light can be selected, the UV detector tends to perform as a universal detector. There are exceptions: the UV detector will not readily detect hydrocarbons, aliphatic alcohols or other substances that do not have a UV chromophore that will absorb light in the wavelength range already defined.

The sensor cell consists of a short cylindrical tube having two terminating flat quartz windows and radial connections at either end for the column eluent to enter and to leave. To reduce band dispersion, the volume of the cell is usually limited to between 2 and 5 μL. The UV light passes axially through the end windows and falls on a photoelectric cell (or array), the output from which is conveyed to an appropriate amplifier and thence to a recorder or data acquisition system.

The cell must be carefully designed to reduce peak dispersion that would result from the natural parabolic velocity profile of the mobile phase as it passes through the cell. A diagram of a typical UV absorption sensor cell is shown in **Figure 1**. The inlet and outlet conduits are designed to produce secondary flow and break up the parabolic velocity profile that causes peak dispersion. Mobile phase enters the cell at an angle, and is directed at the cell window. As a consequence, the stream of mobile phase must virtually reverse its direction to pass through the cell, producing a strong radial flow which disrupts the Newtonian flow.

The same situation is arranged to occur at the exit end of the cell. The flow along the axis of the cell must reverse its direction to pass out of the port that is set at an angle to accomplish the same effect. By employing this type of cell geometry, dispersion in the cell resulting from viscous flow can be practically eliminated.

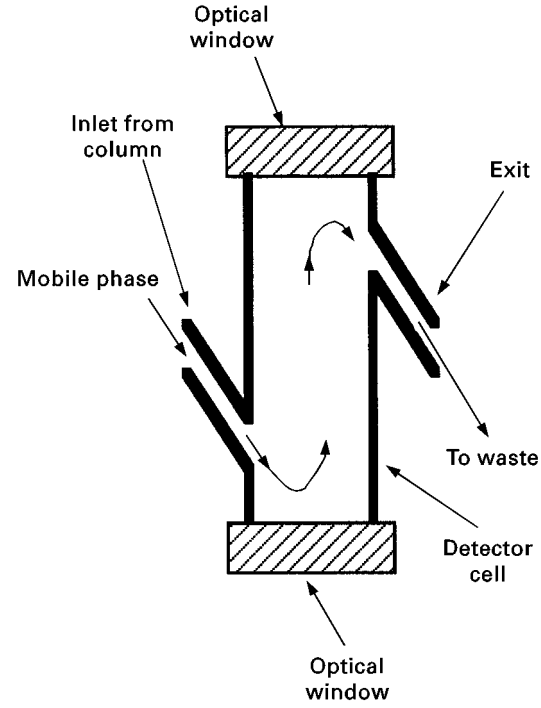

Figure 1 A simple UV detector sensor cell.