Abstract The use of absorbance and fluorescence spectrometers as liquid chromatography detectors is described. These detectors offer sensitive detection that can also identify the peaks in a chromatogram. Many examples, mainly from the separation of polycyclic aromatic hydrocarbons, are given that show the selectivity and usefulness of these detectors.

Keywords HPLC detection · UV absorbance · Fluorescence · Full spectrum · Diode array detector

Introduction

UV absorbance and fluorescence are useful types of spectrometry for some types of molecules. The two measure changes in the energy levels of the molecular electrons. This usually involves the $\pi$-bonding or lone pair electrons in the molecule, but higher energy (lower wavelength) transitions involving $\sigma$-bonding electrons can also be seen.

Absorbance spectrometry measures the energy when electrons go from the ground state to an excited state, while fluorescence measures the process of that energy change and the one that results when the energy is lost through emitted light. Fetzer and Biggs have reviewed the uses of these types of detectors in environmental analyses [1]. The specific advantages of full-spectrum UV and fluorescence detection were shown by examples relating to detection of the polycyclic aromatic hydrocarbons (PAHs).

Photodiode-array-based absorbance detector (DAD)

The photodiode-array-based absorbance detector, usually abbreviated as PDA or DAD, has been used in many applications since it was introduced over two decades ago. This detector collects the complete absorbance spectrum of the column eluent. For this review, the abbreviation DAD will be used. The general principles and operation of the DAD have been reviewed by Huber and George [2]. This book is also a good primer on the use of the DAD in fields such as clinical, pharmaceutical, environmental, polymer, and biotechnology analyses.

In the DAD, a beam from the light source is passed through the flow cell. Any light absorbed by a compound flowing through is attenuated following normal Beer-Lambert law behavior at all wavelengths. The resulting light is diffracted and focused on a row of photodiodes (the array). Each photodiode covers a small spectral range and the incident intensity affects its electronic output. With the collection of the UV spectra of the column eluent, peak identification and quantification can both be done.

Certain compound classes, such as the PAHs, are ideal for DADs. The PAH spectra are both intense and have many bands that are identifiable. Small differences in isomeric structure lead to great differences in band patterns. This difference arises from the different arrangements of the $\pi$ electrons in the isomers. Figures 1 and 2 show the absorbance spectra for similar PAHs, with each having only one or two rings more on the core structure, dibenzo[cd,lm]perylen. The second pair are isomers. Although the two spectra are similar, there are major differences that are characteristic. For other compound classes, these differences will be less, because the presence of sulfur, oxygen, nitrogen, and other heteroatoms makes the electronic distributions less distinct and individualistic. In the dynamic mode used with HPLC, the limit for useful diagnostic spectra is around 0.01 AU.

Other structural factors, such as methyl or other alkyl substitution can also be seen in the DAD spectra. A single...
alkyl-group attachment shifts the absorbance spectrum of the parent compound 1–2 nm upwards. Although additional alkyl substitution shifts the spectrum higher, it is not additive. The presence of a fused saturated ring usually causes a shift of 5–10 nm. To observe such small shifts in the spectra, the DAD must have sufficient resolution.

The DAD monitors all wavelengths within a range. This makes it a much more of a universal detector than the older single-wavelength designs. In the latter devices, major components might be assumed to be minor ones if the wavelength used was not at one of the stronger absorbance bands. If the wavelength was in a region where there was no absorbance, it would not be seen. For example, benzene absorbs strongly at 254 nm. This wavelength was often used for alkylbenzenes. Alkyl or saturated ring substituents will shift the spectrum to a very different maximum. The compound 1,2,3,4,5,6,7,8,9,10,11,12-dodecahydrotriphenylene [3] is a benzene ring with three saturated rings. Its most-intense absorbance maximum is at 273 nm, a shift of 19 nm (Fig. 3). This band has a molar absorptivity of about 325. At 254 nm, the absorptivity is vastly different and is only 110. Equal amounts of the two at 254 nm would show as two peaks at 6.3 to 1.

DADs acquire spectra from a low wavelength of 190 nm to an upper wavelength as high as 800 nm. Most compounds absorb appreciably in the UV and lower visible range (350–500 nm; compounds appearing yellow or orange will absorb in this range). Metal complexes, dyes, and certain PAHs, are among the few compounds that absorb above 500 nm.

DADs can also detect unexpected compounds. Two examples will be given. In the separation of the higher fullerenes, Fetzer and Gallegos [4] used non-aqueous reversed-phase HPLC for a fullerene extract. All previous reports of the composition of fullerenes, only had C-60 (~90%) and C-70 (~10%). These authors observed a series of small peaks in the DAD output after these two expected ones. These were proved to be higher fullerenes of C-76, C-78, C-80, C-82, and C-84. Figure 4 shows the HPLC-DAD chromatogram with these small peaks appearing on the much more intense tail of the C-70 peak, with the corresponding mass spectrum is given in Fig. 5. Other workers later characterized them using similar techniques [5, 6, 7].

Another example was the unexpected discovery of a new eight-ring PAH [8, 9, 10]. A certain eight-ring PAH was prepared by synthesis. This target PAH was isolated by reversed-phase HPLC with a DAD. After isolating the desired compound, a dichloromethane mobile phase was used to clean off the column. After a short time, the effluent showed a peak, whose spectrum was obviously a PAH (Fig. 6). This was a strongly-retained isomer of the target PAH, whose capacity factor was around two orders of magnitude greater than the other isomer (the upper spectrum of Fig. 1).

The substituent effect described earlier for the alkylbenzenes leading to shifts to higher wavelengths is more generic and is applicable to all compound types and a large number of substituents. The classic Woodward-Hoffman additivity “rules” can estimate the shifts. These