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Photodiode Array Detection in Clinical Applications; Quantitative Analyte Assay Advantages, Limitations and Disadvantages

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1. Introduction

1.1 Optical spectroscopy

Study of the electromagnetic radiation by matter, as related to the dependence of these processes on the wavelength of the radiation. More recently, the definition has been expanded to include the study of the interactions between particles such as electrons, protons, and ions, as well as their interaction with other particles as a function of their collision energy. Spectroscopic analysis has been crucial in the development of the most fundamental theories in physics, including quantum mechanics, the special and general theories of relativity, and quantum electrodynamics.

Spectroscopic techniques have been applied in virtually all technical fields of science and technology. One of the most famous kinds of spectroscopy, optical spectroscopy is used routinely to identify the chemical composition of matter and to determine its physical structure.Spectroscopic techniques are extremely sensitive. Single atoms and even different isotopes of the same atom can be detected among 10^{20} or more atoms of a different species. Isotopes are all atoms of an element that have unequal mass but the same atomic number. Isotopes of the same element are virtually identical chemically. Trace amounts of pollutants or contaminants are often detected most effectively by spectroscopic techniques. Because of this sensitivity, the most accurate physical measurements have been frequency measurements.

Spectroscopy now covers a sizable fraction of the electromagnetic spectrum. The table (1) summarizes the electromagnetic spectrum over a frequency range of 16 orders of magnitude. Spectroscopic techniques are not confined to electromagnetic radiation, however. Because the energy E of a photon (a quantum of light) is related to its frequency ν by the relation $E = h\nu$, where h is Planck's constant, spectroscopy is actually the measure of the interaction of photons with matter as a function of the photon energy. In instances where the probe particle is not a photon, spectroscopy refers to the measurement of how the particle interacts with the test particle or material as a function of the energy of the probe particle.

Electromagnetic radiation is composed of oscillating electric and magnetic fields that have the ability to transfer energy through space. The energy propagates as a wave, such that the crests and troughs of the wave move in vacuum at the speed of 299,792,458 metres per second.

Electromagnetic phenomena		
Gamma rays (γ rays)	$<5 \times 10^{-12}$	$>6 \times 10^{19}$
X-rays	5×10^{-12}–1×10^{-8}	3×10^{16}–6×10^{19}
Ultraviolet	1×10^{-8}–4×10^{-7}	7×10^{14}–3×10^{16}
Visible light	4×10^{-7}–7×10^{-7}	4×10^{14}–7×10^{14}
Infrared	8×10^{-7}–1×10^{-3}	3×10^{11}–4×10^{14}
Microwaves, Radar	1×10^{-3}–1	3×10^{8}–3×10^{11}
Television waves	1–10	3×10^{7}–3×10^{8}
Radio waves	10–1,000	3×10^{5}–3×10^{7}

Table 1. Frequency and wavelength domain of electromagnetic radiations

The decomposition of electromagnetic radiation into its component wavelengths is fundamental to spectroscopy. Evolving from the first crude prism spectrographs that separated white light into its constituent colours, modern spectrometers have provided ever-increasing wavelength resolution. Large-grating spectrometers are capable of resolving wavelengths as close as 10^{-3} nanometre, while modern laser techniques can resolve optical wavelengths separated by less than 10^{-10} nanometre. The frequency with which the electromagnetic wave oscillates is also used to characterize the radiation. The product of the frequency (ν) and the wavelength (λ) is equal to the speed of light (*c*); *i.e.,* $\nu\lambda = c$. The frequency is often expressed as the number of oscillations per second, and the unit of frequency is hertz (Hz), where one hertz is one cycle per second.

Spectroscopy is used as a tool for studying the structures of atoms and molecules. The large number of wavelengths emitted by these systems makes it possible to investigate their structures in detail, including the electron configurations of ground and various excited states.

Spectroscopy also provides a precise analytical method for finding the constituents in material having unknown chemical composition. In a typical spectroscopic analysis, a concentration of a few parts per million of a trace element in a material can be detected through its emission spectrum.

Production and analysis of a spectrum usually require the following: (1) a source of electromagnetic radiation, (2) a disperser to separate the light into its component wavelengths, and (3) a detector to sense the presence of light after dispersion (See Figure 1). The apparatus used to accept light, separate it into its component wavelengths, and detect the spectrum is called a spectrometer. Spectra can be obtained either in the form of emission spectra, which show one or more bright lines or bands on a dark background, or absorption spectra, which have a continuously bright background except for one or more dark lines.

1.1.1 Optical detectors

The principal detection methods used in optical spectroscopy are photographic (*e.g.,* film), photoemissive (photomultipliers), and photoconductive (semiconductor). Prior to about 1940, most spectra were recorded with photographic plates or film, in which the film is placed at the image point of a grating or prism spectrometer. An advantage of this technique is that the entire spectrum of interest can be obtained simultaneously, and low-intensity spectra can be easily taken with sensitive film.

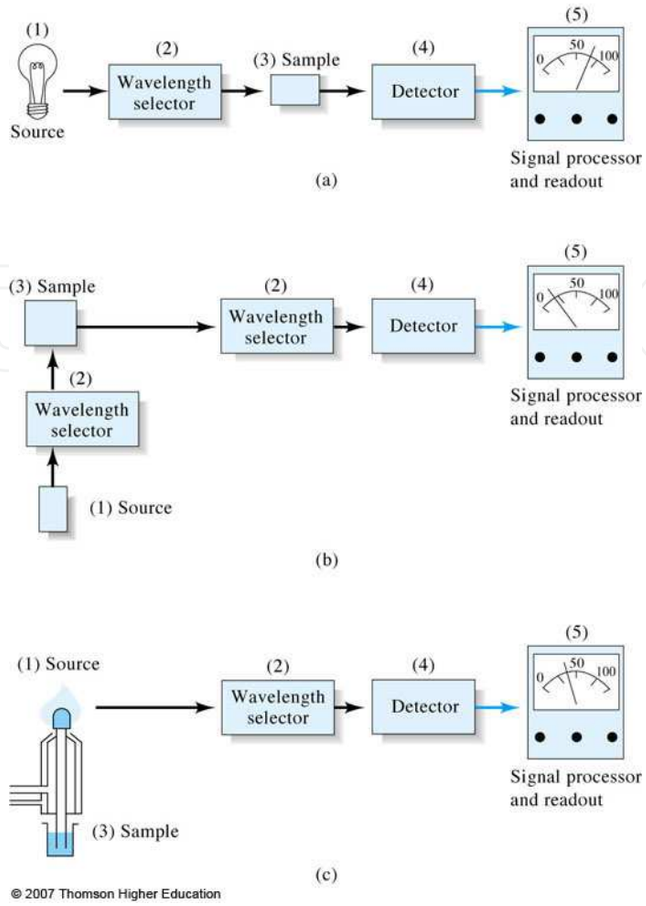

Fig. 1. Components of Optical Instruments. The generic spectrometer, (a) Molecular absorption , (b) Molecular emission and (c) Atomic absorption

Photoemissive detectors have replaced photographic plates in most applications. When a photon with sufficient energy strikes a surface, it can cause the ejection of an electron from the surface into a vacuum. A photoemissive diode consists of a surface (photocathode) appropriately treated to permit the ejection of electrons by low-energy photons and a separate electrode (the anode) on which electrons are collected, both sealed within an evacuated glass envelope. A photomultiplier tube has a cathode, a series of electrodes (dynodes), and an anode sealed within a common evacuated envelope. Appropriate voltages applied to the cathode, dynodes, and anode cause electrons ejected from the cathode to collide with the dynodes in succession. Each electron collision produces several more electrons; after a dozen or more dynodes, a single electron ejected by one photon can be converted into a fast pulse (with a duration of less than 10^{-8} second) of as many as 10^{7} electrons at the anode. In this way, individual photons can be counted with good time resolution.

Other photodetectors include imaging tubes (*e.g.*, television cameras), which can measure a spatial variation of the light across the surface of the photocathode, and microchannel plates, which combine the spatial resolution of an imaging tube with the light sensitivity of a photomultiplier. A night vision device consists of a microchannel plate multiplier in which the electrons at the output are directed onto a phosphor screen and can then be read out with an imaging tube.

Solid-state detectors such as semiconductor photodiodes detect light by causing photons to excite electrons from immobile, bound states of the semiconductor (the valence band) to a state where the electrons are mobile (the conduction band). The mobile electrons in the conduction band and the vacancies, or "holes," in the valence band can be moved through the solid with externally applied electric fields, collected onto a metal electrode, and sensed as a photoinduced current. Microfabrication techniques developed for the integrated-circuit semiconductor industry are used to construct large arrays of individual photodiodes closely spaced together. The device, called a charge-coupled device (CCD), permits the charges that are collected by the individual diodes to be read out separately and displayed as an image.

1.1.2 Multichannel detectors

Multichannel detectors can be used to sense optical and ionizing radiation or convert to an electrical signal an incoming chemical, physical, mechanical, or thermal stimulus. In other words; multichannel detector, can measure all wavelengths dispersed by a dispersing elemnt simultaneously.

The multichannel detector employs a light source that emits light over a wide range of wavelengths. Employing an appropriate optical system (a prism or diffraction grating), light of a specific wavelength can be selected for detection purposes. The specific wavelength might be chosen where a solute has an absorption maximum to provide maximum sensitivity. Alternatively, the absorption spectra of an eluted substances could be obtained for identification purposes by scanning over a range of wavelengths. The latter procedure, however, differs with the type of multichannel detector being used.

There are two basic types of multi–wavelength detector, the *dispersion* detector and the *diode array detector*, the latter being the more popular. In fact, very few dispersion instruments are sold today but many are still used in the field and so their characteristics will be discussed. All multichannel detectors require a broad emission light source such as deuterium or the xenon lamp, the deuterium lamp being the most popular.

The two types of multichannel detectors have important differences. In the dispersive instrument, the light is dispersed before it enters the sensor cell and thus virtually monochromatic light passes through the cell. However, if the incident light is of a wavelength that can excite the solute and cause fluorescence at another wavelength, then the light falling on the photo cell will contain the incident light together with any fluorescent light that may have been generated. It follows, that the light monitored by the photocell may not be monochromatic and light of another wavelength, if present, would impair the linear nature of the response. This effect would be negligible in most cases but with certain fluorescent materials the effect could be significant. The diode array detector operates quite a differently. Light of *all wavelengths* generated by the deuterium lamp is passed through the cell and then dispersed over an array of diodes. Thus, the absorption at discrete groups of wavelengths is continuously monitored at each diode. However, light falling on a discrete diode may not be derived solely from the incident light but may contain light generated by fluorescence excited by light of a shorter wavelength.

The ideal multichannel detector would be a combination of both the dispersion system and the diode array detector. This arrangement would allow a true monochromatic light beam to pass through the detector and then the transmitted beam would itself be dispersed again onto a diode array. Only that diode sensing the wavelength of the incident light would be used for monitoring the transmission. Under some circumstances, measurement of

transmitted light may involve fluorescent light and the absorption spectrum obtained for a substance may be a degraded form of the true absorption curve. In this way any fluorescent light would strike other diodes, the true absorption would be measured and accurate monochromatic sensing could be obtained.

In a multichannel dispersive detector light from the deuterium lamp is collimated by two curved mirrors onto a holographic diffraction grating. The dispersed light is then focused by means of a curved mirror, onto a plane mirror and light of a specific wavelength is selected by appropriately positioning the angle of the plane mirror. Light of the selected wavelength is then focused by means of a lens through the flow cell. The exit beam from the cell is then focused by another lens onto a photocell, which gives a response that is some function of the intensity of the transmitted light. The detector is usually fitted with a scanning facility that allows the spectrum of the solute contained in the cell to be obtained. There is an inherent similarity between UV spectra of widely different types of compounds, and so UV spectra are not very reliable for the identification of most solutes.

A usual use of multichannel choice is to enhance the sensitivity of the detector by selecting a wavelength that is characteristically absorbed by the substance of interest. Conversely, a wavelength can be chosen that substances of little interest in the mixture do not adsorb and, thus, make the detector more specific to those substances that do.

Multichannel dispersive detectors provids adequate sensitivity, versatility and a linear response. But, it has mechanically operated wavelength selection and requires a stop/flow procedure to obtain spectra. In contrast, the *diode array detector* has the same advantages but none of these disadvantages.

Find some important multichannel detector on the list below.

- Photodiode Array (PDA)
  - Semiconductors (Silicon and Germanium) (see Figure 3)
    - Group IV elements
    - Formation of holes (via thermal agitation/excitation)
    - Doping
      - n-type: Si (or Ge) doped with group V element (As, Sb) to add electrons.
        As: $[Ar]4S^2 3d^{10} 4p^3$
- p-type: Doped with group III element (In, Ga) to added holes
  In: $[Kr]5S^2 4d^{10} 5p^1$ (see Figure 4)
- coupled device (CCD)
- vidicon

1.1.3 Photodiode array detectors

A photodiode array is a linear array of several hundred light sensing diodes light ranging from 128 to 1024 – and even up to 4096 having a thousand phototubes, for every different wavelength. The design of this kind of machine is somewhat different and simpler. (Figures 2-4) Light passes through the sample first. Then it hits the monochromator, and then it is dispersed onto the photodiode array.

This multichannel detector makes an ideal sensor for an entire spectrum in a UV-VIS dispersive spectrophotometer. With that application, newer arrays have been made with adjacent diodes 25.6 mm long and spaced 25 mm on centers.

A polychromatic beam from the source is irradiated onto the inlet slit of the polychromator after passing through the sample compartment. The polychromator disperses the narrow

band of the spectrum onto the diode array. The photodiode converts light into electrical signals and temporarily stores them. These signals are then read out as time-series signals via the output line by sequentially turning on the switch array connected to each photodiode with address pulses generated from the shift register.

A silicon photodiode consists of a reversed biased *pn* junction formed on a silicon chip. A photon promotes an electron from the valence bond (filled orbitals) to the conduction bond (unfilled orbitals) creating an electron(-) - hole(+) pair. The concentration of these electron-hole pairs is dependent on the amount of light striking the semiconductor. Spectral resolution limited by size of diode.

PDA detectors are useful in both research and quality assurance laboratories. In the research laboratory, the PDA provides the analyst with a variety of approaches to the analysis. In the quality assurance laboratory, the PDA provides several results from a single run, thereby increasing the throughput of the HPLC.

PDA detection offers the following advantages:

- **Peak measurement at all wavelengths:**
  In methods development, detailed information about the detector conditions required for the analysis may not be known. When a variable wavelength detector is used, a sample must often be injected several times, with varying wavelengths, to ensure that all peaks are detected. When a PDA detector is used, a wavelength range can be programmed and all compounds that absorb within this range can be detected in a single run.
- **Determination of the correct wavelengths in one run:**
  After all peaks have been detected, the maximum absorbance wavelength for each peak can be determined. A PDA detector can collect spectra of each peak and calculate the absorbance maximum.
- **Detection of multiple wavelengths:**
  A PDA detector can monitor a sample at more than one wavelength. This is especially useful when the wavelength maxima of the analytes are different. Wavelengths can be selected to analyze each compound at its highest sensitivity.
- **Peak purity analysis:**
  It is difficult to determine component purity from a chromatogram. However, a PDA detector can analyze peak purity by comparing spectra within a peak. A pure peak has matching spectra throughout the peak (at all wavelengths).
- **Positive peak identification:**
  In liquid chromatography, peak identification is usually based on relative retention times. When a PDA detector is used, spectra are automatically collected as each peak elutes. The PDA software compares the spectra with those stored in a library to determine the best fit matches; this method increases the likelihood of correctly identifying peaks.
- **Scan spectrum very quickly:**
  entire spectrum in <1 second
- **Provides single beam.**
- **Powerful tool for studies of transient intermediates in moderately fast reactions.**
- **Useful for kinetic studies.**
- **Useful for qualitative and quantitative determination of the components exiting from a liquid chromatographic column.**

In addition to above points, there are many major advantages of diode array detection. In the first, it allows for the best wavelength(s) to be selected for actual analysis. This is

particularly important when no information is available on molar absorptivities at different wavelengths.

The second major advantage is related to the problem of peak purity. Often, the peak shape in itself does not reveal that it actually corresponds to two or even more components. In such a case, absorbance rationing at several wavelengths is particularly helpful in deciding whether the peak represents a single compound or, is in fact, a composite peak.

As already mentioned, a special feature of some variable wavelength UV detectors is the ability to perform spectroscopic scanning and precise absorbance readings at a variety of wavelengths while the peak is passing though the flow cell. Diode array adds a new dimension of analytical capability to liquid chromatography because it permits qualitative information to be obtained beyond simple identification by retention time.

In absorbance rationing, the absorbance is measured at two or more wavelengths and ratios are calculated for two selected wavelengths. Simultaneous measurement at several wavelengths allows one to calculate the absorbance ratio. Evaluation can be carried out in two ways:

In the first case, the ratios at chosen wavelength are continuously monitored during the analysis: if the compound under the peak is pure, the response will be a square wave function (rectangle). If the response is not rectangle, the peak is not pure.

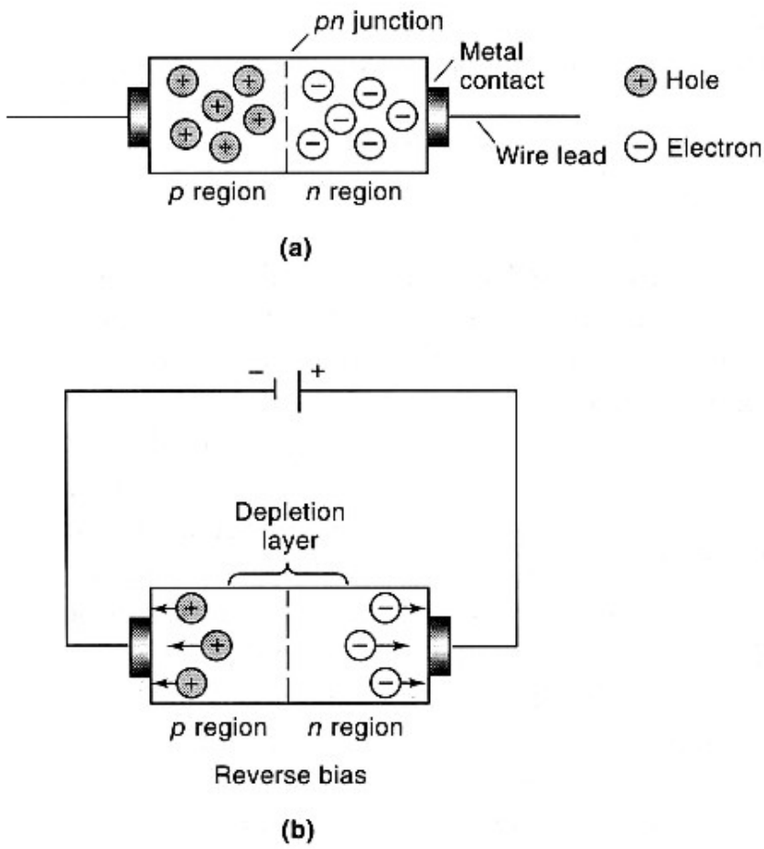

Fig. 2. (a) Schematic of a silicon diode, (b) Formation of depletion layer which prevents of flow of electricity under reverse bias [Skoog & Leary,1992].

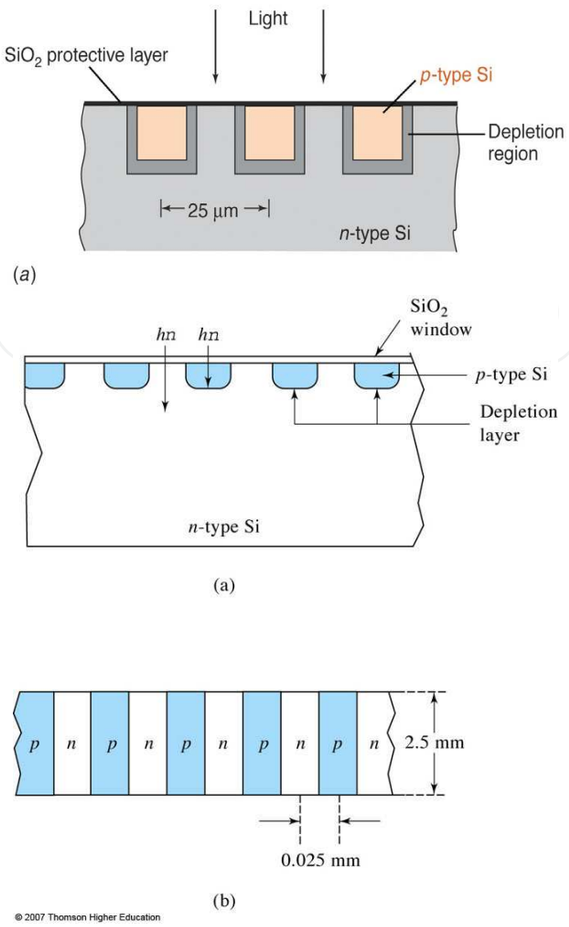

Fig. 3. (a) n-type and (b) p-type photodiode array.

Photodiode array (PDA) detectors scan a range of wavelengths every few milliseconds and continually generate spectral information. Wavelength, time, and absorbance can all be plotted.

In methods development, detailed information about the detector conditions required for the analysis may not be known. When a variable wavelength detector is used, a sample must often be injected several times, with varying wavelengths, to ensure that all peaks are detected. When PDA detectors provide three-dimensional information that allows an accurate assessment of peak identity, purity, and quantitation in a single run. Software support for PDA detectors includes peak purity and spectral library search functions to help determine peak homogeneity and identity.

1.1.4 Photodiode array applications

Spectrometers have developed in many ways since the introduction of simple spectrophotometers which were commercially available from the mid 1950's. Such improvements have enabled us to use PDA type UV-Vis. spectrophotometers.

The scope and performance of conventional single channel detector type UV-Vis spectrophotometers were found to be somewhat limited. This encouraged a search for novel techniques which could be applied to the development of UV-Vis. spectrophotometers.

Dispersed light is focused directly onto the detector array, saving considerable time and greatly reducing instrument complexity. The combination of dispersing element and detector array is employed in most spectrophotometers today.

1.1.4.1 UV-Vis spectroscopy

The introduction of multichannel detectors such as the linear photodiode array (PDA), charge coupled device (CCD) and vidicon enabled new detection systems to be developed for UV-Vis spectrophotometers and encouraged the rapid development of polychromators from the 1970s [Talmi, 1975,1982].

As was expressed earlier a polychromator is an enhanced monochromator which it is accomplished by electronic scanning of the multichannel detector.Multichannel detectors such as the photodiode array, charge coupled device or vidicon are usually flat and are best used with a dispersing arrangement which yields a flat focal plane. Under optimum conditions, they can detect as many wavelengths simultaneously as their number of individual diodes, resolution elements or pixels. Stray light and background per element are negligible because they are arrays and they have very low dark currents.

PDA, on the other hand, is more suited for applications where the light level is relatively high. Because in the PDA the photon saturation charge is greater than CCD so the detection range of PDA is larger than CCD. Furthermore, PDA delivers lower noise than CCD. So it PDA was recommend in applications where higher output accuracy is needed.

This multichannel detector having numbers of elements ranging from 128 to 1024 and even up to 4096. It makes an ideal sensor for an entire spectrum in a UV-VIS dispersive spectrophotometer.

A polychromatic beam from the source is irradiated onto the inlet slit of the polychromator after passing through the sample compartment. The polychromator disperses the narrow band of the spectrum onto the diode array. The photodiode converts light into electrical signals and temporarily stores them. These signals are then read out as time-series signals (see Figure .4).

A spectrum for the whole wavelength range should be acquired for best results. The correlation between wavelengths and particular detector channels in a polychromator facilitates nearly simultaneous measurement of the intensities of the various wavelengths.

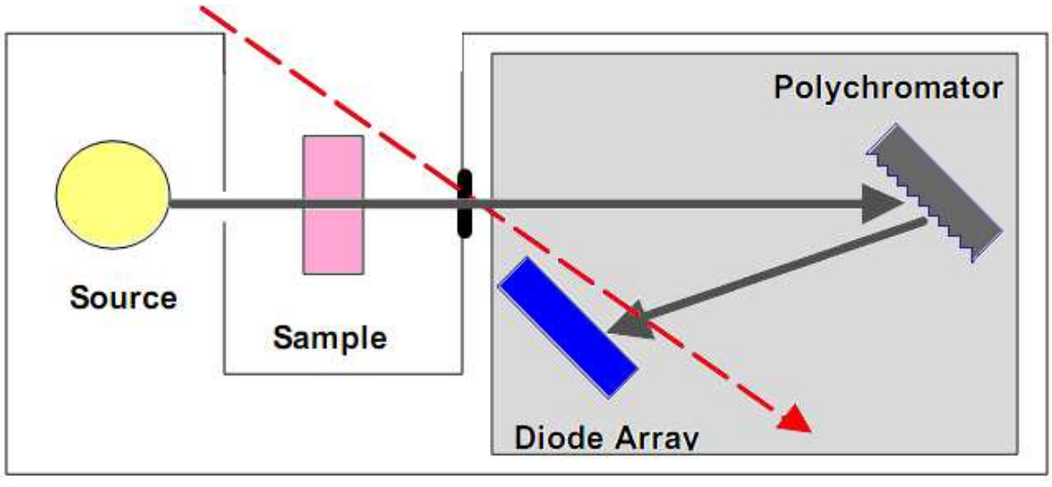

Fig. 4. Schematic of a photodiode array spectrophotometer.

The conventional UV-Vis. spectrophotometer only has one detector. But data for many wavelengths can be acquired with the photodiode array spectrophotometer simultaneously since there are several hundred or a thousand detectors present.Fast spectral acquisition makes diode array spectrophometers the first choice for measurement of fast chemical reactions and kinetics study of materials.

The duration and intensity of illumination determine both the final S/N ratio and the exposure interval needed to acquire a spectrum. This interval is also the integration time for the signal. A longer integration time allows a higher S/N since the signal will be larger and noise averaged more completely towards zero.

There is no Integration function in the conventional UV-Vis spectrophotometer which accumulates the signal. For example, the total required time will be 1000 sec. for 1000 data points and it takes 1 sec. to measure one datum. In this case, all 1000 data have the same signal to noise ratio (S/N). But in a PDA instrument which has a 1000 photodiode array, 1000 data points can be measured in 1 sec. and it would take 1/1000 sec. to achieve the same result obtainable in 1 sec. in a conventional instrument. Therefore, when the same sample is measured for 1000 sec in a PDA instrument, the signal is accumulated and is 1000 times greater than when measuring for 1 sec. The noise will be 1000. This means that the S/N ratio is improved by 1000.

This resulting benefit of fast data acquisition is termed Felgett's Advantage or Multichannel Advantage.

In a conventional UV-Vis spectrophotometer mechanical movement is required to select a specific wavelength. But a photodiode array UV-Vis spectrophotometer acquires data at each wavelength by electrical scanning. In this way, the wavelength reproducibility of a PDA instrument is much better than the conventional mechanical scanning UV-Vis spectrophotometer. In addition, a photodiode array type spectrophotometer has a reversed optic structure which minimizes stray light problems, a serious issue in conventional UV-Vis spectrophotometers.

On the other hand, a PDA is a solid-state device and is more secure and reliable than a PMT (photomultiplier tube). Furthermore, a polychromator avoids the variations in optical performance with wavelength and time that are introduced in a scanning monochromator by moving the grating. Indeed, in a polychromator no mechanical movement is required except perhaps the opening of a shutter at the entrance slit.

The Spectroscopy methods which are used of PDA can be divided into 3 sections: mass spectrometry, atomic spectroscopy and molecular spectroscopy. The applications of PDA for all 3 sections have been growing steadily. UV-Vis, FT-IR, Fluorescence, Raman and NIR spectroscopy instruments are in the molecular group. UV-Vis, is the largest category in this section. UV-Vis spectroscopy finds applications not only in traditional chemistry but also in newer fields such as pharmaceuticals & life science, environment, agriculture, energy and the petrochemical Industry.

1.1.4.2 Photodiode array and HPLC

The great importance of diode-array detection in HPLC can be characterized by the fact that this is solely the subject of an excellent book edited by Huber and George [Huber & George, 1993].

The most important advantage of the diode-array UV detector over conventional multiwavelength UV detectors is the speed of scanning the spectra. Using the reversed optics of the diode-array spectrophotometer enables all points in the spectrum to be

measured simultaneously on the array of fixed photodiodes. The speed of scanning the spectrum is thus determined by the speed of data acquisition. In modern diode-array UV detectors equipped with powerful computers the time necessary to take the full spectrum from 190 to 600 nm can be reduced to as short as about 10 msec. This speed is more than sufficient in the overwhelming majority of cases in pharmaceutical analysis when the half-band width of peaks separated by HPLC is usually in the order of 1 min and it is only very rarely in the order of 1-10 sec in fast HPLC systems and especially in capillary electrophoresis where the peaks are in general narrower.

The quality of the UV spectrum of the separated impurities obtained by the diode-array detector is influenced by several of photodiodes. For example, the number of diodes in a DAD of the HPLC instrument is only 205 while in the other it is 1024. If the spectrum has fine structure, better quality spectra are obtainable with the latter. In addition to this the quality of the spectra of especially the low level impurities greatly depends on the baseline noise. This can be reduced by using a light source with high intensity, by selecting a suitable reference wavelength (which is as close to the cut-off wavelength of the separated analyte as possible and a suitable slit width. Generally speaking the sensitivity of the new generation of diode-array detectors is much higher than that of the older ones.

There are three main areas within drug impurity profiling where the advantages of diode-array detectors can contribute to the success of the HPLC (CE) analysis (see Figures 5-7).

(a)Peak purity determination. The determination of peak homogeneity is an integral part of the protocol in the validation of any kind of HPLC (and CE) analysis of pharmaceuticals. In the course of impurity profiling studies it is especially important to check the peak of the main component for its homogeneity from the simple and most widely used absorbance ratio method [Drouen et al.,1984; Wilson et al.,1989] to more sophisticated deconvolution, spectral suppression, spectrum subtraction and other chemometric methods[Huber & George, 1993]. If any kind of peak in-homogeneity is found (impurity on the leading or tailing edges of the main peak or fused impurity peaks, conveniently demonstrated in the three-dimensional mode) the diode-array spectra themselves furnish further information for the identification of the unresolved impurities.

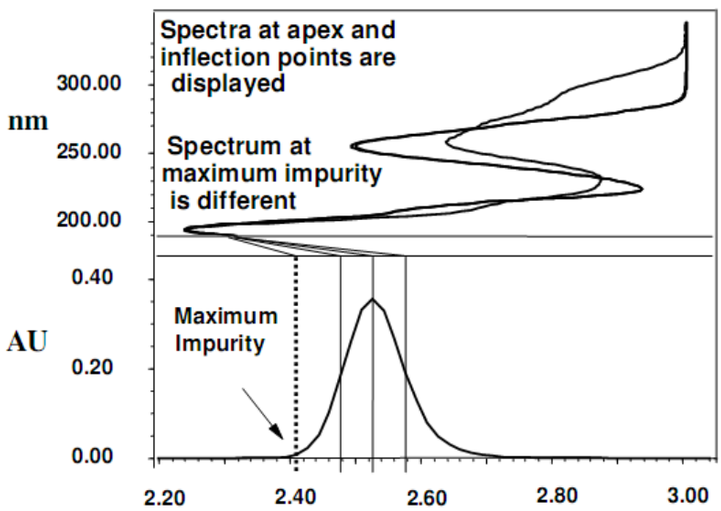

Fig. 5. Peak purity measurement

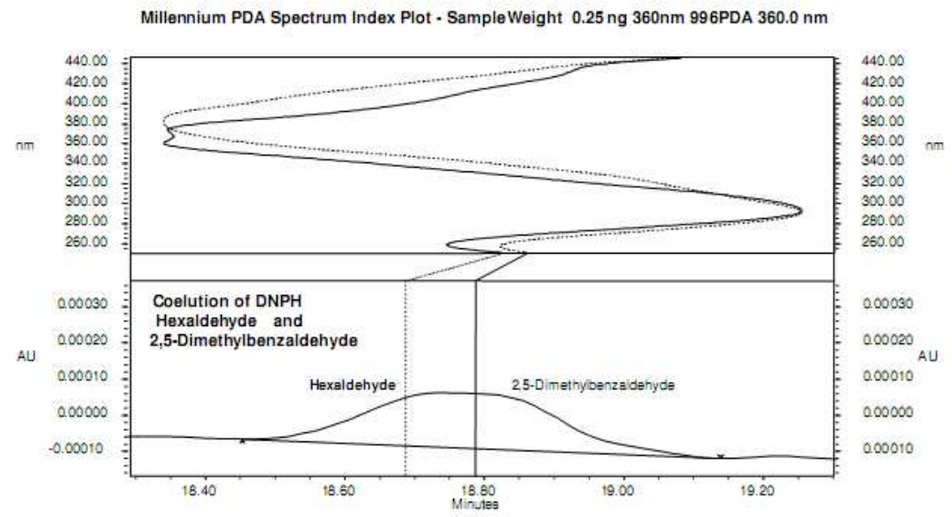

Fig. 6. Maximum impurity detection

(b) Spectral matching. Matching the diode-array spectra of components separated by HPLC with those taken by computer search from spectral libraries is a widely used method [Huber & George, 1993] especially in toxicological analysis . This approach is of limited value in drug impurity profiling since it is unlikely that impurities of especially new drugs are included in spectrum libraries. However, matching the diode-array spectra of the separated impurities with standard materials can greatly support the identification of the impurities on the basis of retention matching.

(c) Structure elucidation of the separated impurities. It is reasonable to begin the search for the structure unknown impurity separated by HPLC or CE with drawing as many conclusions from its diode-array UV spectrum as possible.

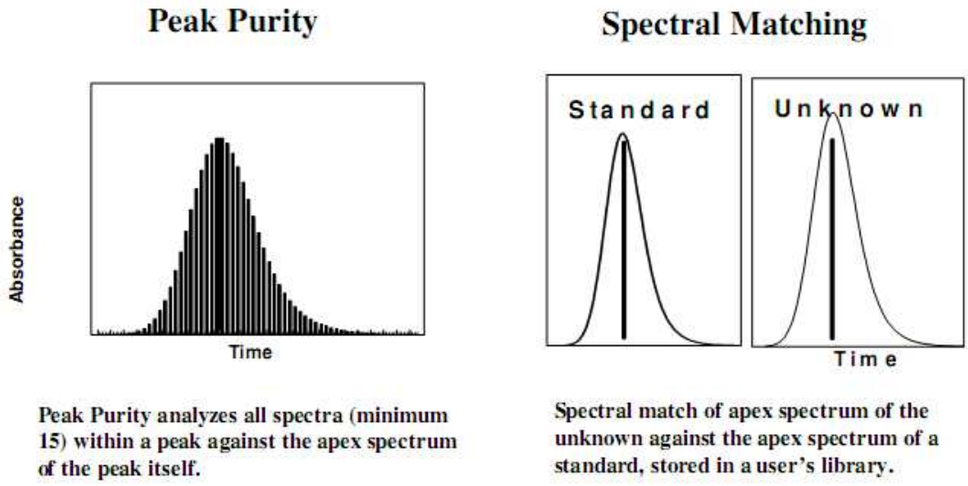

Fig. 7. Determination of peak purity

The short-wavelength parts of the (diode-array) UV spectra can be subject of several distorting effects, moreover even false maxima can occur. In addition to this, short-

wavelength UV bands can originate from different chromophoric functional groups and for this reason they are of limited value in the structure elucidation of organic compounds. As a consequence of these factors it is a prerequisite of drawing useful conclusions from the UV spectrum of an impurity that it should have at least one maximum above 210-220 nm.
Another limitation is that the difference between the structures of the drug material and the impurity should be at or near the chromophoric part of the molecule in order that the difference between their spectra can be of diagnostic value in the structure elucidation of the impurity. For example, the chromophoric group of various steroids is the 4-ene-3-oxo group with an absorption maximum around 240 nm. As it will be shown later, the position of this band is influenced by substituents in the B and C ring of the steroid nucleus but by no means by substituents at C-17. For this reason various esters of 17-hydroxy-4-ene-3-oxo steroids (testosterone, 19-nortestosterone, 17-hydroxyprogesterone, etc.) cannot be differentiated on the basis of their UV spectra.
HPLC with photodiode array detection (HPLC-PDA or HPLC-DAD) is regularly employed for substance identification in the context of Systematic Toxicological Analysis [Koves,1995; Gaillard & Pépin,1997; Herre & Pragst,1997]. With HPLC-PDA the most important parameters in identifying a compound are its retention time and its UV spectrum. Critics of the method often question the specificity of UV detection because of poorly structured spectra and broad absorption bands. Therefore a systematic investigation into the selectivity of PDA detection was carried out by analyzing large numbers of UV spectra with respect to their correlation with chemical structure.
For data analysis the following tools are needed:

1. A spectra library ; the library is embedded into the chromatography software in a way that spectral similarity is compared nm by nm and a “hit list” is returned to the operator.
2. A database of retention times and specific peak areas.
3. A database of all molecular structures with an ability for substructure searches.
4. A structural database of all registered chromophores.

As an alternative to Mass Spectrometers, absorbance detectors (including PDA) are much less expensive and relatively simple to use. LC-DAD is a fast and robust method for screening biological samples in conjunction with a library search algorithm to quickly identify those samples that require confirmatory testing. Numerous methods for using LC-PDA as a screening method have been published and were recently reviewed by Pragst et al. [Pragst et al.,2004]. Because a PDA detector can collect an entire spectrum at each time point in a chromatogram, the data are information rich and more selective than single wavelength chromatograms. Herzler et al. [Herzler et al.,2003] showed that PDA data could be used to selectively identify abused substances in spectrochromatograms based on comparison to a library of over 2500 “toxicologically relevant” substances. Their method relied on the calculation of a ‘similarity index’ (related to the correlation coefficient) to determine the similarity between a spectrum in an unknown chromatogram and a library spectrum. In addition to spectral matching, a relative retention time was also used to identify the substances of interest.

1.1.4.3 Medical chemistry applications of HPLC-PDA

High performance liquid chromatography (HPLC) with photodiode array detection has been proved to be the demanded method of systematic analysis for unknown drugs in biological sample because of separation efficiency, sensitivity, flexibility and identification

potential. HPLC can be an easy way of quantitation as well. Ultraviolet spectra acquired with photodiode array detector together with retention data are used to identify unknown or suspected drugs and metabolites in various biological material. These analytical systems are suitable for toxicological examinations of forensic cases, acute poisonings, drug abuse. They are convenient to subsequent monitoring of serum drug levels during treatment of intoxication as well.

High-performance liquid chromatography coupled with diode array detection (HPLC-DAD) has been widely used as a powerful means for the analysis of multi-component medicines, which can provide a UV chromatogram and comprehensive data about the compounds in complex mixtures [Han et al.,2007; Su etal.,2010; Wei et al.,2010; Zhang et al.,2010]. This technology facilitates identification of unknown components in the matrices system remarkably with high sensitivity and accuracy.

Photodiode array (PDA) detectors record light absorption at different wavelengths and can provide spectra of the analytes. This is useful in identifying unknowns. Mass spectrometry (MS) is a better detector for unknowns. It gives an unambiguous molecular weight of an analyte and provides structural information. When coupled with CE or HPLC, MS can separate co-eluting analytes with different mass to charge ratios. But the Mass spectrometer is an expensive instrument and the possibility of using it is not available in all laboratories. Of course, if possible HPLC/ESI-MS/UV-DAD analysis gives the best sensitivity [Cuyckens& Claeys,2002; Beretta et al.,2009; Christiansen et al.,2011].

The potentials and limitations of high-performance liquid chromatography-photodiode array detection are highlighted in respect to its use in the analysis of different biological matrices followed by the identification of unknowns. The logical analytical approach used in clinical and forensic toxicology, vital for the identification of one or more toxic substances as a cause of intoxication, is largely based on both simple and fast "general unknown screening" methods which cover most relevant drugs and potentially hazardous chemicals. In this field of systematic toxicological analysis, a literature overview shows that HPLC can play a substantial role. Both column packing material and eluent composition have their impact on intra- and inter laboratory reproducibility. In view of the sometimes different retention characteristics of various HPLC columns, several possibilities are addressed to enhance the discriminating power of primary retention parameters. The advantages of photodiode array detection as compared to UV detection have been of paramount importance to the success of HPLC in toxicological analysis. Dedicated libraries with spectral information and searching software are powerful tools in the process of identification of an unknown substance. In the present section, these aspects are also verified in a number of real cases.

HPLC-DAD used as a general unknown screening tool should cover as many drugs and toxicants as possible, but should be also very selective, sensitive and reliable. Liquid chromatography is used in forensic laboratories for numerous applications including examination of drugs. LC with photodiode array detection (PDA) is a hybrid technique which can provide complete UV-visible spectral information on a given peak in a chromatogram, enabling determinations of peak purity to be made, and identification of unknown peaks to be assigned by library searches of spectral information in combination with retention behavior. These are valuable features normally associated with gas chromatography-mass spectrometry. The additional information available on each peak makes LC-PDA a particularly attractive technique for the forensic laboratory where higher levels of certainty are often demanded in test results. This paper reviews some of those

applications for LC-PDA in the forensic sciences, including drug screening, drug and pharmaceutical analysis, idenfication of pesticides, fungi, quality control testing and profiling of cosmetics, street drugs and profiling of other complex mixtures. The practical and technical limitations of the technique are explored and its place in the hierarchy of methods available in forensic laboratories is evaluated [Proença et al.,2003; Madej et al.,2003; Proenc et al.,2004; Nieddu et al., 2007; Es'haghi et al.,2010; ; Vosough et al.,2010].

HPLC-DAD offers many advantages in terms of specificity, sensitivity, speed and ruggedness. The data produced, comprising both retention behavior and absorption spectra of eluting chemical entities, result in an identification power at low cost and with widened availability through many laboratories. In addition, the examples showed a great versatility in application fields and excellent quantitative potential. The fast progress in DAD detector technology, computer and software power and HPLC packing material quality have led to an exponential rise of the number of reports on the use of HPLC-DAD. The advent of routine use of HPLC-MS will probably promote HPLC as a viable if not better alternative to GC-MS.

We examined that combined with a sample preparation method; HPLC-PDA can be easy achieved to very low detection limits [Es'haghi et al., 2009, 2010]. In a research, we used of direct suspended droplet microextraction (DSDME) method, based on a three-phase extraction system which is compatible with HPLC-PDA for determination of ecstasy; MDMA (3,4methylendioxy-N-methylamphetamine) in human hair samples. After the extraction, pre-concentrated analyte was directly introduced into HPLC for further analysis. In concentration range between 1.0 and 15,000 ng mL^{-1} calibration curve is drowned. Linearity was observed with r = 0.9921 for analyte. Limit of detection (LOD) were calculated as the minimum concentration providing chromatographic signals three times higher than background noise. Limit of quantification (LOQ) was estimated as the minimum concentration preparing chromatographic signals ten times higher than background noise. Thus, LOD obtained was 0.1 and LOQ was 1.0 ng mL^{-1} too [Es'haghi et al., 2010].

In the other work we successfully used of DSDME method combined with HPLC-PDA for determination of low-residue benzodiazepine, diazepam and lorazepam, in the environmental water samples [Es'haghi et al., 2009, 2009]. After the optimized extraction conditions, the suspended micro-droplet is withdrawn by a HPLC microsyringe, injected to and analyzed by HPLC-DAD. Method was evaluated and enrichment factor 839.8, linearity range from 25 to 5000 ng mL^{-1} with an average of relative standard deviation (n=5) 5.62% for diazepam using a photodiode array detector were determined. HPLC-PDA has good matches with complex matrices such as hair.

A method combining liquid–liquid–liquid microextraction and automated movement of the acceptor and donor phases (LLLME/AMADP) with ion-pair HPLC/DAD has been developed to detect trace levels of chlorophenols in water [Lin etal.,2008] . The extracted chlorophenols, present in anionic form, were then separated, identified, and quantitated by ion-pair high-performance liquid chromatography with photodiode array detection (HPLC/DAD). For trace chlorophenol determination using HPLC/DAD, the chlorophenolate anion provides a better ultraviolet spectrum for quantitative and qualitative analyses than does uncharged chlorophenol. The proposed method was capable of identifying and quantitating each analyte to 0.5 ng mL^{-1} , confirming the HPLC/DAD technique to be quite robust for monitoring trace levels of chlorophenols in water samples.

HPLC/DAD could simultaneously detect UV absorptions at multiple wavelengths and extract the UV spectra of separated analytes in a chromatogram. Absorbance measurements at the band maxima of UV spectra obey the linear Beer's law more accurately than

measurements off the band maxima, and UV spectra of the separated analytes can be utilized to identify target analytes in HPLC/DAD. Accordingly, each extracted chlorophenolate anion after ion-pair liquid chromatography separation was quantitated by the maximum adsorption of its own red shift characteristic band, and each target chlorophenlate anion was identified by its own red shift characteristic band as well as its enhanced B band. The chlorophenols were determined under selected experimental conditions to assess repeatability, linearity, coefficient of determination, and detection limit.

A HPLC-DAD method for drug screening in plasma were developed by M. A. Alabdalla [Alabdalla,2005]. This analytical method extracted and tested a number of drugs of different classes. The method included; an acidic and basic Solid Phase Extraction (SPE) of plasma with C18 cartridges, a gradient elution of a modified cyano column with acidic buffer/acetonitrile eluent and a photodiode array ultraviolet (UV) detection. The drug screening procedure applied used retention index and UV spectral data for the identification of compounds, may be appropriate in particular laboratory settings.

Continuous administration of polyphenols from aqueous rooibos (Aspalathus linearis) extract ameliorates dietary-induced metabolic disturbances in hyperlipidemic mice was studied by HPLC-DAD and introduced by R. Beltrán-Debón et al. [Beltrán-Debón et al., 2011]. In this biological matrices and they could find good results.

In a recent study neurons from the olfactory system of the fish crucian carp, Carassius carassius L. were used as components in an in-line neurophysiologic detector (NPD) to measure physiological activities following the separation of substances by high-performance liquid chromatography (HPLC). The skin of crucian carp, C. carassius L. contains pheromones that induce an alarm reaction in conspecifics. Extra-cellular recordings were made from neurons situated in the posterior part of the medial region of the olfactory bulb known to mediate this alarm reaction. The nervous activity of these specific neurons in the olfactory bulb of crucian carp was used as an in-line neurophysiologic detector. HPLC was performed with a diode array detector (DAD) [Brondz et al.,2004].

UV spectral detection was performed at 214, 254 and 345 nm, and scans (190–400 nm) were collected continuously. This system enabled the selection of peaks in the chromatogram with fish alarm pheromone activity. Neurophysiologic detectors (NPDs) in-line with diode array detectors (DADs) are able to provide the physiologically active substances and their spectral characteristics.

Li-wei Yang et al. were developed a method using high-performance liquid chromatography–photodiode array detection (HPLC–DAD) for the quality control of Hypericum japonicum thunb (Tianjihuang), a Chinese herbal medicine. For the first time, the feasibility and advantages of employing chromatographic fingerprint were investigated for the evaluation of Tianjihuang by systematically comparing chromatograms with a professional analytical. The results revealed that the chromatographic fingerprint combining similarity evaluation could efficiently identify and distinguish raw herbs of Tianjihuang from different sources. The effects resulted from collecting locations; harvesting time and storage time on herbal chromatographic fingerprints were also examined [Yang et al.,2005].

1.1.4.4 Photo diode array detector in kinetic study

In kinetic experiments, transient optical absorption is recorded versus time to evaluate rate constants related to the species under investigation. In addition, the recording of a spectrum sometimes becomes necessary in order to identify the species. In most cases, the spectrum is constructed from point-to-point recordings of kinetic curves at selected wavelengths. This procedure is time consuming, and becomes boring especially at long recording times in the

second and minute time domain. The use of a device, which enables the recording of a complete spectrum, can be very helpful as it reduces experiment time remarkably. Unwanted side effects, such as photolysis during long recording times, can also be prevented. The application of optical multichannel analyzers which use either a linear charge coupled device (CCD) or a linear photodiode array (PDA) in kinetic experiments was reported by some laboratories [Hunter et al .,1985; Sedlmair et al.,1986; Johnson et al.,1994]. The advantage of using such a detector is the ability to immediately record a complete spectrum from UV to IR with one measurement.

The PDA detector has the ability to record a spectrum over a large range of wavelengths. The uniformity of the analyzing light intensity over the whole range is important because the dynamics and the sensitivity of the measurements depend largely on the intensity. The spectral distribution of the analyzing light, as recorded by the multichannel detector is shown in Figure .8.

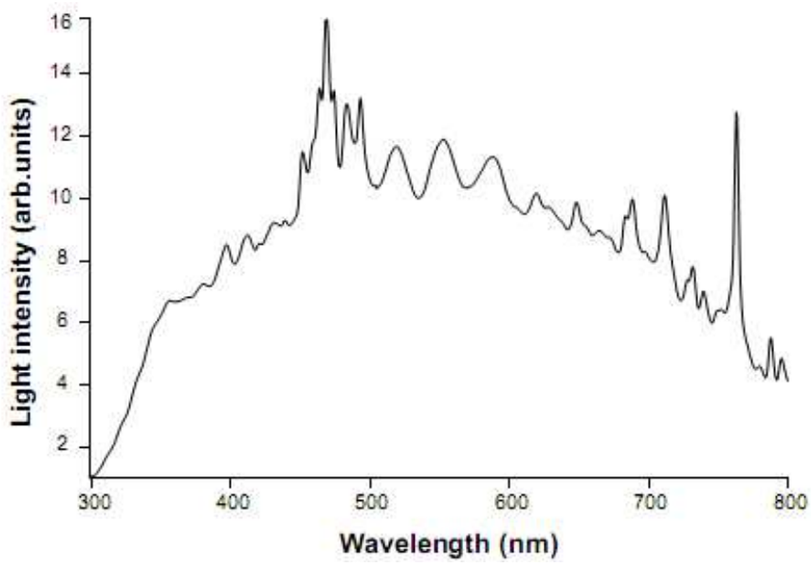

Fig. 8. Light intensity vs. wavelength of an xenon lamp, recorded by the multichannel detector.

The source of the analyzing light is an xenon lamp. The light intensity is attenuated tenfold as compared to kinetic experiments. Although, the recorded intensity of the analyzing light decreases drastically below 350 nm, a spectral range from 300 to 800 nm can be covered. Below 300 nm, recording should be accomplished in small segments and with the help of band-pass filters in order to adjust for the reduced level of analyzing light and for the decreased sensitivity of the detector, and, in addition, to avoid scattered light effects. The measurement depends largely on proper focusing of the light path, i.e., how well the lamp arc is imaged onto the diode array.

Each spectrum is the average of some (for example five) individual measurements; each irradiation consists of a train of ten pulses. The interval between the recordings of the individual spectra or between the pulses in each pulse train was set to zero. The recording at time zero, i.e. before irradiation, shows a straight line. The change in absorption increases with increasing irradiation. In general, kinetic trace scan be constructed from the recorded spectra at selected wavelengths. Similar to the construction of spectra from kinetic traces [Janata,1994].

At measurements in the UV region, Cerenkov emission is a common problem at short measuring times. The intensity of the Cerenkov emission increases with decreasing wavelength and can be much larger than the kinetic signal itself, but probably will not exceed the intensity of the analyzing light. Although this apparatus makes data at longer time scale available, overdriving of the photodiodes and long recovery times are conceivable.
The use of an optical multichannel detector consisting of a linear diode array embedded in the instrumentation for kinetic spectroscopy, as well as the highlights of the computer program used for controlling the gathering and the evaluation of data are described. Complete spectra can be recorded and irradiation can be triggered according to a preset timetable. Due to the read-out time of the photodiode array and the time required by the computer to control the experiment, this apparatus is suitable for application starting in the millisecond time domain and extending up to very long time periods.

1.1.4.5 Chemometrics investigations using photo diode array detection

Chemometrics is a statistical approach to the interpretation of patterns in multivariate data. When used to analyze instrument data, chemometrics often results in a faster and more precise Assessment of composition of a product or even physical or sensory properties. For example, composition of drugs can be quickly measured using LC and chemometrics. Food properties can also be monitored on a continuous basis. In all cases, the data patterns are used to develop a model with the goal of predicting quality parameters for future data. The two general applications of chemometrics technology to predict a property of interest; and to classify the sample into one of several categories (e.g., good versus bad, Type A versus Type B versus Type C etc.). Chemometrics can be used to speed methods development and make routine the use of statistical models for data analysis. Keeping in view of the complexity of the chromatographic fingerprint and the irreproducibility of chromatographic and spectral instruments and experimental conditions, several chemometric approaches such as variance analysis, peak alignment, correlation analysis and pattern recognition were employed to deal with the chromatographic fingerprint. Many mathematical algorithms are used for data processing in chemometric approaches. The basic principles for this approach are variation determination of common peaks/regions and similarity comparison with similarity index and linear correlation coefficient. Similarity index and linear correlation coefficient can be used to compare common pattern of the chromatographic fingerprints obtained. In general, the mean or median of the chromatographic fingerprints under study is taken as the target and both are considered to be reliable [Brereton,1987].
The rapid scanning detectors, as diode array detection, present an alternative technology for rapid, multi-wavelength detection in HPLC. If hyphenated chromatography is further combined with chemometric approaches, clear pictures might be developed for chromatographic fingerprints obtained. A chemical fingerprint obtained by hyphenated chromatography, out of question, will become the primary tool for quality control of medicines.
The full UV-Vis spectrum became accessible as a three-dimensional (3D) data matrix (A, A, t). Data are available in the time, concentration and wavelength domains. This allows the simultaneous use of more than two wavelengths for detection or for the full application of detector information to the analytical problem by means of available chemometric techniques to data from second-order bilinear instruments, as chromatographic and excitation-emission data.
As an alternative to MS, absorbance detectors (including PDA) are much less expensive and relatively simple to use. LC-DAD is a fast and robust method for screening biological samples

in conjunction with a library search algorithm to quickly identify those samples that require confirmatory testing. Numerous methods for using LC-DAD as a screening method have been published and were recently reviewed by Pragst et al. [Pragst et al., 2004]. Because a DAD can collect an entire spectrum at each time point in a chromatogram, the resultant data are information rich and more selective than single wavelength chromatograms.

For the above reasons could be adopted PDA detectors with the various chemometric methods to match spectra contained within a spectrochromatogram to a library.

In a research, triply coupled diode array detection high performance liquid chromatography mass spectroscopy was applied to a complex mixture of at least eight chlorophyll degradation products. Derivatives were employed to determine parts of the chromatogram of composition one. Mass selection was performed on the mass spectroscopic data. Principal components analysis was performed on both the raw and simultaneously normalised/standardised data; three dimensional projections of the data were obtained and compared to conventional two dimensional graphs. Angular plots between diode array loadings characteristic of individual compounds and scores of the diode array data were described. In mass spectra, angular plots between loadings characteristic of individual compounds and the remaining diagnostic masses revealed further mass spectral structure [Zissis et al.,1999].

Liquid chromatography–chemometric methods [LC-Partial least squares (LC-PLS), LC-principle component regression (LC-PCR) and LC-artificial neural network (LC-ANN)] were developed for the determination of anomalin (ANO) and deltoin (DEL) in the root by Alev Tosun et al.[Tosun et al.,2007]. Firstly, chemometric conditions were optimized by testing different mobile phases at various proportions of solvents with various flow rates in different wavelengths by using a normal phase column to obtain the best separation and recovery results. As a result, a mobile phase consisting of n-hexane and ethyl acetate (75:25 v/v) at a constant flow rate of 0.8 mL min $^{-1}$ on the at ambient temperature were found to be the optimal chromatographic conditions for good separation and determination of ANO and DEL in samples. Multi-chromatograms for the concentration set containing ANO and DEL compounds in the concentration range of 50–400 ng mL^{-1} were obtained by using a diode array detector (DAD) system at selected wavelength sets, 300 (A), 310 (B), 320 (C), 330 (D) and 340 (E). Three LC-chemometric approaches were applied to the multichromatographic data to construct chemometric calibrations. As an alternative method, traditional LC at single wavelength was used for the analysis of the related compounds in the plant extracts. All of the methods were validated by analyzing various synthetic ANO–DEL mixtures. After the above step, traditional and chemometric LC methods were applied to the real samples consisting of extracts from roots and aerial parts of analytes.

In a recent research, metabolism disorders in Kunming mice induced by two tumor cells were characterized. Metabolic fingerprint based on high performance liquid chromatography-diode array detector (HPLC-DAD) was developed to map the disturbed metabolic responses. Based on 27 common peaks, principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used to distinguish the abnormal from control and to find significant endogenous compounds which have significant contributions to classification. The tumor growth inhibition ratios of Taxol groups were used to validate the predictive accuracies of the PLS-DA models. The predictive accuracies of PLS-DA models for tumors model groups were 97.6 and 100%, respectively. Nine and seven of two models tumors were discovered, including uric acid and cytidine. In addition, the correlations between relative tumor weights and chromatographic data were significant ($p < 0.05$). Investigations on the stability and precision of the established metabolic

fingerprints demonstrate that the experiment is well controlled and reliable. This work was shown that the platform of HPLC-DAD coupled with chemometric methods provides a promising method for the study of metabolism disorders [Sun et al., 2011].

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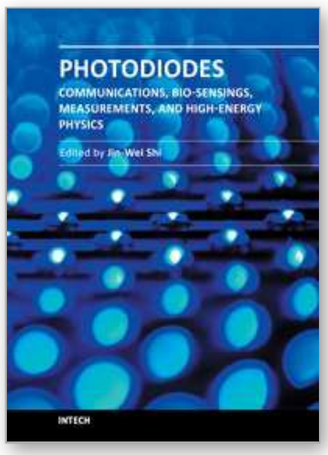

Photodiodes - Communications, Bio-Sensings, Measurements and High-Energy Physics

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This book describes different kinds of photodiodes for applications in high-speed data communication, biomedical sensing, high-speed measurement, UV-light detection, and high energy physics. The photodiodes discussed are composed of several different semiconductor materials, such as InP, SiC, and Si, which cover an extremely wide optical wavelength regime ranging from infrared light to X-ray, making the suitable for diversified applications. Several interesting and unique topics were discussed including: the operation of high-speed photodiodes at low-temperature for super-conducting electronics, photodiodes for bio-medical imaging, single photon detection, photodiodes for the applications in nuclear physics, and for UV-light detection.

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