



Review

Analytical devices based on light-emitting diodes – a review of the state-of-the-art



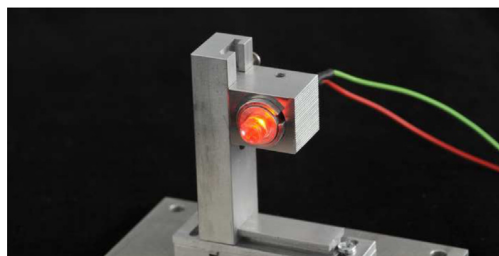
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HIGHLIGHTS

- A review of the current status of the use of LEDs in analytical devices is given.
- Fundamental aspects with relevance to quantitative measurements are discussed.
- A broad overview of the reported applications is given.

GRAPHICAL ABSTRACT



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ABSTRACT

A general overview of the development of the uses of light-emitting diodes in analytical instrumentation is given. Fundamental aspects of light-emitting diodes, as far as relevant for this usage, are covered in the first part. The measurement of light intensity is also discussed, as this is an essential part of any device based on light-emitting diodes as well. In the second part, applications are discussed, which cover liquid and gas-phase absorbance measurements, flow-through detectors for chromatography and capillary electrophoresis, sensors, as well as some less often reported methods such as photoacoustic spectroscopy.

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Abbreviations: LED, light-emitting diode; IR, infrared; LDR, light-dependant resistor; PMT, photo-multiplier tube; PEDD, paired emitter detector diodes; AU, absorbance unit; ADC, analogue-to-digital convertor.

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1. Introduction

Light-emitting diodes became commercially available in the 1960s [1]. The early devices emitted red light and this was followed by the introduction of LEDs of shorter wavelengths, i.e. orange, yellow and green. Blue LEDs were first sold in the mid-1980s, near-UV (ultraviolet) devices at 370 nm in about 2000 and only in recent years deep-UV devices, i.e. with wavelengths below 320 nm, have become available. Currently LEDs with wavelengths down to 240 nm can be obtained commercially. The shorter the wavelength, the higher the energy, which requires the more difficult creation of semiconductor junctions with higher bandgaps. Along with the move to lower wavelengths has also been a trend to higher intensities. Parallel to these developments occurred an extension to longer wavelengths, so that now devices with wavelengths up to about 4.6 μm in the mid-IR (infrared) range are also available commercially.

The general applications of LEDs are manifold. Prominent is their use as indicating lights for electronic circuitry, but increasingly LEDs are also used for lighting applications, in particular in form of white light emitting devices (which either contain a fluorescent compound to achieve wide band emission or a combination of LED substrates of different colours). Invisible near-IR LEDs are used for remote controls and automatic door openers, and also, along with semiconductor lasers, for fibre optic telecommunications. Near-UV-LEDs are used for examination of banknotes for forgery and invisible owner markings. Deep-UV-LEDs have potential for use in disinfection. The brightest visible LEDs now have such intensity that radio amateurs were able to demonstrate audio communications links based on the transmission of the modulated light of single LEDs over free space through distances of 83 km during daytime [2] and 167 km at night [3].

The development of applications of LEDs in analytical chemistry naturally has followed the progression in wavelengths and intensity. The first report on an analytical LED device appears to

be a publication by Flaschka et al. [4]. The photometric instrument was based on a red LED and a phototransistor [4]. Analytical devices based on blue LEDs were first reported in about 1986 [5], while first instruments employing deep-UV-LEDs were reported about 5 years ago [6,7]. The development of deep-UV-LEDs is very significant for analytical applications as most organic molecules absorb in this range, but not in the near-UV or visible wavelength regions. LEDs in the IR range have been found to be most useful for the detection of small gas molecules such as CO_2 . Many of the reported devices based on LEDs are low cost alternatives to commercial instruments, but on the other hand, due to the inherent properties of the LEDs, well designed devices are capable of delivering the highest performance, often surpassing that of instruments based on conventional light sources.

The use of LEDs in analytical devices has been reviewed repeatedly. As the field is very wide, most of these have been focussed on specific areas, but there are some more general overviews. Dasgupta et al. in 1993 [8] wrote a review concerning absorption measurements in flow through detectors based on LEDs, and again in 2003 [9] covered the topic with the inclusion of fluorescence and spectroelectrochemical techniques. Capitán-Vallvey and Palma have summarized developments in handheld and portable analytical instruments based on optical sensing [10]. Their review does not exclusively cover LED-based devices, but due to their utility for battery operated portable instruments, they feature very prominently. O'Toole and Diamond have reviewed sensors and sensing devices based on absorbance measurements with LEDs in 2008 [11]. During the preparation of this article a further review by Dasgupta and coauthors has appeared which is focussed mainly on the developments of absorbance and fluorescence measurements in liquids with LEDs over the period from 2008 to 2013 [12]. The interested reader is advised to also consult this complementary publication.

This review is intended to give a broad overview of the state-of-the-art concerning analytical uses of LEDs. Fundamentals are

covered as far as relevant for the basic construction of analytical devices, without delving into the underlying semiconductor physics. Given the breadth of the subject it naturally cannot comprehensively cover all publications in the field. Often publications are cited as examples to illustrate a point, but it should not be taken to imply that there are not other equally valid publications as well. Not discussed are applications of LEDs in chemistry in general, such as for decomposition of molecules or curing epoxies. Also not discussed are purely physical sensing and detection with LEDs, nor are laser diodes.

2. Fundamental considerations

2.1. Characteristics of LEDs

A photograph of an LED in the most common package is shown in Fig. 1. This is referred to as a 5 mm, or T-1 3/4, package. The latter denotation stems from an earlier standard for tungsten filament lamps and corresponds to its diameter as a multiple of 1/8th of an inch [13]. The light originates from the semiconducting material contained in a reflective cup in the centre on top of one of the connecting leads. Note that there is a very thin wire contacting the semiconducting material from the top, which is connected to the second lead. The entire assembly is encapsulated in an epoxy resin. Different forms are available, such as miniature types, surface mount variants, or special high power versions. All forms have in common a much higher degree of robustness and miniaturization than the common light sources used in analytical instruments, which are usually either incandescent light bulbs or discharge lamps. The dome of the standard package shown in Fig. 1 forms a lens and disperses the light. LEDs with narrow or wider beam angles are available according to their intended purposes. For analytical applications it is usually not desirable to have the light dispersed but it should be focussed or collimated. It is possible to cut off the dome, followed by polishing of the surface, in order to get closer access to the emitter, but one must be careful not to break the top connecting wire in the process. Such polished LEDs are well suited for coupling to optical fibres, in particular the plastic fibres with 1 mm diameter. The latter corresponds to a readily available standard. However, tight focussing, e.g. for detection on capillaries or coupling into narrower optical fibres, or good collimation of the light, is difficult as the emitting substrates are not point sources. Ball lenses may be the best option for narrow focussing.



Fig. 1. Photograph of a blue LED in the common 5 mm package. The emitting chip is contained in the well on top of the pin on the right. The dome acts as a lens to disperse the light.

Other advantages are high current efficiency and therefore low heat production, which in turn reduces intensity drifts. Further advantageous features compared to conventional light sources used in analytical instruments are long lifetimes, and low cost. All these attributes make them attractive for use in analytical instrumentation, in particular for low power portable devices. A further important aspect, which strongly distinguishes LEDs from conventional light sources employed in analytical instruments, is the restriction of the emission to bands of typically 30 nm in width. This wavelength limitation further improves the efficiency of the light source. Note that, in contrast, laser diodes have much narrower emission lines. The emission spectra of a selection of LEDs covering the range from about the shortest currently available wavelength of 255 nm to about 900 nm are shown in Fig. 2. LEDs for wavelengths much further into the IR are also available. The emission bands are usually clean, but deep-UV-LEDs may also show spurious emissions at longer wavelength which would have to be considered in the design of a device. The wavelength selectivity is often the most important ground for choosing LEDs as light sources in instrumentation as this can eliminate a costly monochromator, which is generally needed with conventional lamps, and few other light sources with inherent wavelength restriction are available. Generally a good match of the emission bands of the LEDs with the broad absorbance bands of molecules can be obtained. On the other hand, of course, such a fixed emission band can also be a limitation. This can, to some extent, be overcome by the use of switchable LED arrays [14–16], while still benefitting from the other advantages of the LEDs. In recent years white LEDs have also become available. Some of these devices are based on the combination of three different substrates emitting the three primary colours. By balancing the intensities the impression of white light is achieved. A second type of white LEDs is based on the combination of a short wavelength emitter with an embedded fluorescent material to achieve emission over a wider wavelength range. Both types appear white to the human eye, but show gaps in the emission spectra. If used for analytical instruments a wavelength dispersive element would normally be required, as with a more conventional light source, and one would have to be aware of the gaps in the emission.

A further advantage is the possibility for fast switching. This feature is made use of in infrared remote controls and fibre optic communications, and bandwidths well into the MHz range are

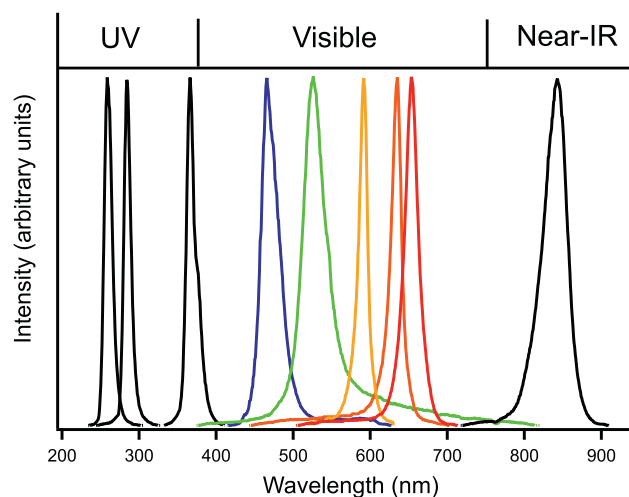


Fig. 2. Emission spectra of a selection of LEDs from the deep UV to the near IR. UV: 255 nm, 280 nm, 365 nm. Visible: blue at 464 nm, green at 516 nm, amber at 590 nm, red at 635 nm, dark red at 645 nm. IR: 850 nm.

possible [1]. This characteristic is also of interest when employing LEDs in analytical instrumentation as this means that for modulation of the light intensity it is not necessary to employ mechanical beam choppers, as is usually the case with conventional light sources due to their slow response, but this can be done with electronic oscillators. This is useful for lock-in amplification, e.g. for the suppression of ambient light interference, or for lifetime measurements in fluorescence.

Light-emitting diodes are non-linear devices and show the typical logarithmic current vs. voltage characteristics (i/V -curve) of a diode shown in Fig. 3. This means that they cannot be operated directly from a voltage source as slight changes in the supply voltage or drifts in the junction voltage (such as those due to temperature changes) can cause not only pronounced changes in output intensity, but may also lead to catastrophic failure of the device. Therefore they should be driven with a constant current source, rather than the common constant voltage source. Usually this is approximated by using a higher voltage and a current limiting resistor. For more precise operation it is better to use an active current control which can easily be achieved with a LM317 voltage regulator integrated circuit in the appropriate configuration, as shown in Fig. 4. Other approaches are possible. The use of such a circuitry is particularly important when the voltage drop across the diode is very high, as is the case for deep-UV-LEDs (about 7 V), and it is driven from a standard low voltage source.

It should be noted, that, while the use of a constant current source reduces the temperature dependence of the output intensity of the LEDs, this effect is far from being perfect as shown in Fig. 5. Measurements for three LEDs of different colours operated with a current limiting resistor and with a constant current source are shown and it can be seen that the temperature stability is only slightly better for the constant current operation. The reason for this must be the fact that with temperature not only the voltage drop across the diode (and with it the current when operated with a current limiting resistor) changes, but also the conversion efficiency of the LED [1]. When good precision is required, it is therefore necessary to compensate for intensity drifts due to temperature changes by using a referenced measuring approach. Stable output intensities can also be achieved by using a servo circuitry which makes use of a photodiode for monitoring. Some commercially available LEDs even include a monitor photodiode in the same case. However, temperature changes may also cause a spectral drift, and for high precision work it may therefore be necessary to control the temperature of the LED with a Peltier (thermoelectric) element. A detailed discussion of the temperature dependence of LEDs and options for compensation can be found in Dasgupta et al. [9].

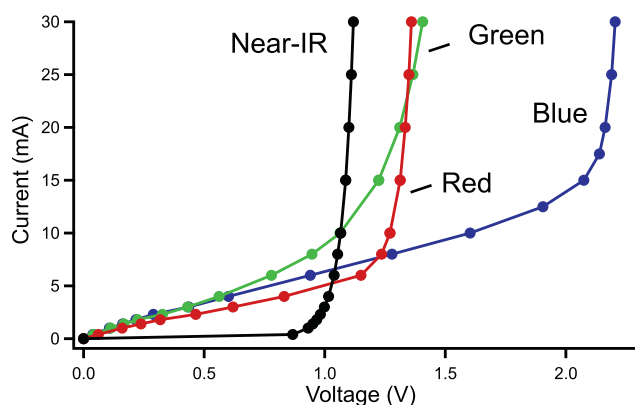


Fig. 3. i - V curves determined for LEDs of different colours.

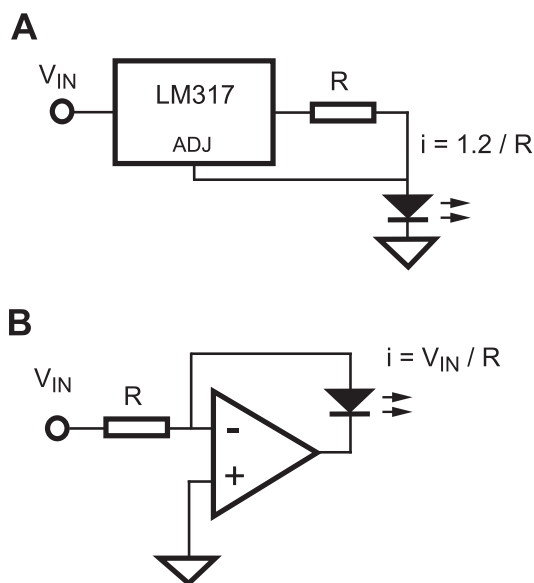


Fig. 4. Constant current circuitries for use with LEDs. (A) Based on the LM317 adjustable voltage regulator integrated circuit. (B) Based on an operational amplifier.

LEDs of vastly different intensities are available. Power LEDs for lighting applications can have optical output powers of up to several Watts (available e.g. from Avago, Philips Lumileds, Kingbright or Osram Opto Semiconductors). However, at the far ends of

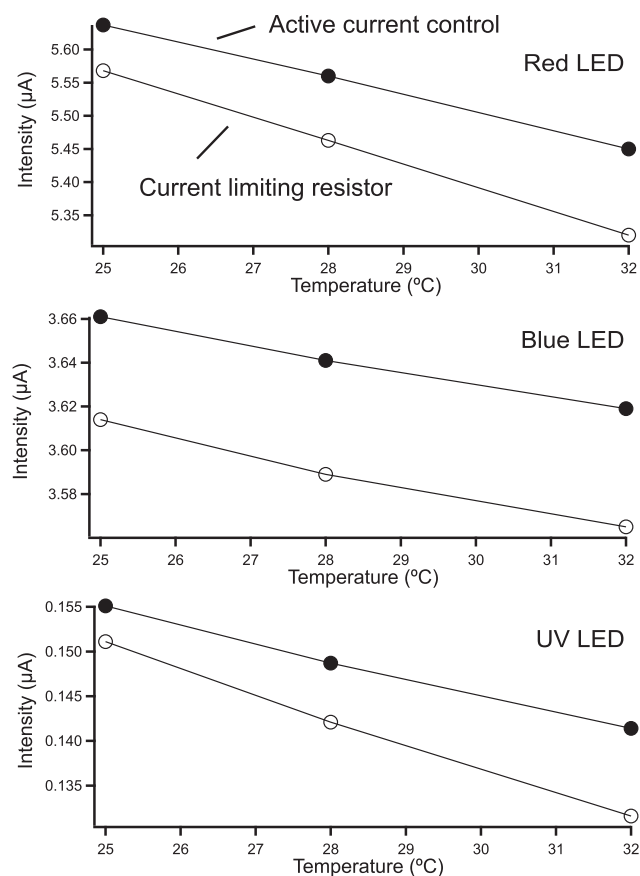


Fig. 5. Temperature dependencies measured for a red, blue and UV (365 nm) LED when operated either with a current limiting resistor from a fixed voltage supply of 5 V, or with a constant current supply according to the circuitry of Fig. 4A.

the spectrum covered by LEDs, i.e. the IR range above 3 μm and the deep UV below 300 nm the maximum available output powers drop to the μW range (see for example www.roither-laser.com). Note that the intensities for visible LEDs are usually specified as luminous intensity (with the units of Candela, cd), which is a measure of the brightness as perceived by the human eye, i.e. it takes into account the dispersion of the light by the body of the LED and the wavelength dependence of the eye sensitivity.

2.2. Detection of light

2.2.1. Detection devices

When using LEDs as light sources in analytical devices it is also necessary to employ detectors for light. The exception are the thermo-optic methods. One of these is photoacoustic sensing, which is discussed in a later section. A discussion of the use of LEDs is not complete without a consideration of the other half of the set-up. In keeping with the simplicity of the LEDs photodiodes based on semiconductor p/n-junctions are usually employed. In these devices an annihilation of charge carriers occurs at the interface, but the absorption of photons releases the charges. The principle is the same as for photovoltaic cells employed for the production of electricity from sunlight. For visible, and immediately adjacent wavelengths Si-photodiodes work best. In construction, photodiodes are very similar to LEDs, they are solid state and small. Well performing devices can be obtained for as little as about 1 euro and these are therefore as inexpensive as the cheapest LEDs. Special devices, in precision cases, with extra low dark current (background signal when no light is present) or specific wavelength sensitivities, will, of course, be more expensive. Standard Si-photodiodes have a maximum in sensitivity close to 1000 nm, but as dictated by the bandgap of the junction a sharp cut-off at 1100 nm. For use in the UV below about 300 nm, special types with UV-transparent windows are needed. Several alternatives are available for the deep-UV range between about 200 nm and 300 nm, based on SiC, GaN, GaP or TiO_2 . For the IR-range photodiodes based on InGaAs, InAs, InSb or HgCdTe are available. They all have different intrinsic spectral sensitivities and must be chosen according to the wavelength range of interest for the application at hand. Some devices have been designed to cover only relatively narrow bands, and may contain optical filters, such as to allow the determination of the intensities of the UV-A, UV-B and UV-C ranges related to sun-light.

When very low intensities must be measured the more complex and more expensive photomultiplier tubes (PMT) may be employed. The combination of LEDs with PMTs is rare, but an example is a detector for capillary electrophoresis based on a deep-UV-LED which was only available with low intensity [17]. In practice the use of PMTs does not have to be difficult as simple modules which include all the necessary electronics are available. Phototransistors also have higher sensitivities than photodiodes, but are not well suited for reproducible intensity measurements. Note, that also light dependant resistors (LDR) can be used to measure light intensity. These are junctionless semiconductor devices which change their resistance in dependence of the light intensity. The performance of these components is inferior to that of photodiodes, due to non-linearity, hysteresis and slow response times, and they should not be used in modern devices.

While photodiodes do not emit light when a current is passed through, it has been known that LEDs also behave like photodiodes [18], although at lower sensitivity than real photodiodes [8]. An intriguing fact is that the sensitivity of LEDs when used as detectors is limited to a rather narrow wavelength range, with a width similar to their emission bands. But note that the sensitivity is shifted to lower wavelengths compared to their emission. The wavelength selectivity has been made use of in the

construction of inexpensive instruments where this is needed, i.e. for sun light spectrometers [18] and crop monitors [19]. Berry et al. have constructed a molecular absorption spectrometer based on a tungsten broadband emitter and an array of different LEDs as detectors to achieve wavelength selectivity [20]. However, when the wavelength selectivity in an analytical device is already determined by the employment of an LED as source, the use of an LED instead of a photodiode for detection has no clear general benefit, in particular when considering that well performing photodiodes can be obtained for a cost as low as that of inexpensive LEDs. For a detailed discussion and a comparison of the performance of LEDs used as detectors vs. photodiodes see [21]. Nevertheless the combination of two LEDs for analytical devices, one used as source, the other as detector, has been reported repeatedly and has been termed PEDD (paired emitter detector diodes) (see for example [22–24]). An interesting application of the use of LEDs for detection, other than for analytical instrumentation, is in bi-directional digital communication over light where the LEDs alternately function as emitters and as detectors [25].

2.2.2. Intensity measurements

A signal may be obtained from photodiodes in different modes of operation. These can be distinguished fundamentally between current measurements (the photocurrent mode) or voltage measurements (the photovoltaic mode) [26]. The photocurrent is proportional to light intensity, while in the photovoltaic mode a logarithmic response is obtained. For quantitative measurements the photocurrent mode is generally preferred as the photovoltaic mode has a poorer precision, mainly caused by a significant temperature dependence. The photocurrent can easily be measured with the help of an operational amplifier in the current-to-voltage convertor (or current follower) configuration as shown in Fig. 6A. The output voltage of the amplifier is then proportional to the light intensity. In the photovoltaic mode shown in Fig. 6B, the diode voltage is measured, usually with an operational amplifier as a buffer to avoid loading, i.e. drawing a significant current from the photodiode. A further, indirect approach is also possible [27]. The photodiode is first reverse biased, that is a voltage is applied in the polarity which is blocked by the device, in order to charge its intrinsic junction capacitance. In the second step the voltage source is disconnected, and the remaining voltage across the device is monitored. As the junction capacitance is discharged by the photocurrent, the time for decay of the voltage is dependant on the light level. While this appears to

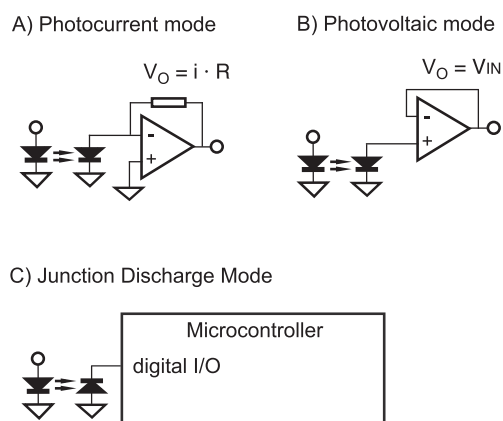


Fig. 6. The different reported modes of measuring light intensities with photodiodes.

be rather complicated, the approach can be implemented easily, essentially in software, using a digital input–output pin of a modern microcontroller without requiring any additional circuitry as illustrated in Fig. 6C. The method has also been employed with LEDs used as detectors [28]. Special photodiodes are also available, such as avalanche photodiodes for low light level measurements, photodiodes with built in amplifiers, and photodiodes with a built in circuitry that gives a light level dependant frequency output for ease of interfacing with a microcontroller. Interference by ambient light can be overcome by intensity modulation of the LED and lock-in detection. This does not need to be complicated, nor expensive, as it can be implemented with a synchronous detector available as an integrated circuit [29,30].

2.2.3. Measurement of absorbance

Analytical measurements based on the absorption of light by the analyte make use of the absorbance (A) parameter, which is directly related to concentration (c), but obtained only indirectly from the light intensities before (I_0) and after (I) passage through the absorbing material as described by the well known Lambert–Beer law:

$$A = \log \frac{I_0}{I} = \varepsilon \times b \times c \quad (1)$$

ε is the molar absorptivity coefficient of the analyte, and b the optical pathlength.

If the light intensities are measured with photodiodes, the intensities (I_0 and I) are converted to currents (i_0 and i), and therefore in practice the absorbance is then given by:

$$A = \log \frac{i_0}{i} \quad (2)$$

The Lambert–Beer law in the common form is, however, strictly only true for monochromatic radiation. For non-monochromatic light sources an extended form of the equation is applicable, which takes into account the variation of the intensity (I_0) of the source, of the detector sensitivity (S) and of the absorptivity (ε) across the wavelength (λ) band of the source:

$$A = \log \frac{i_0}{i} = \log \frac{\int_{\lambda_0}^{\lambda_1} S(\lambda) \times I_0(\lambda) d\lambda}{\int_{\lambda_0}^{\lambda_1} S(\lambda) \times I(\lambda) \times 10^{-\varepsilon(\lambda)bc} d\lambda} \quad (3)$$

As illustrated in Fig. 7, this can in practice lead to more or less strongly pronounced non-linear calibration curves when LEDs are employed for absorbance measurements. The deviation from linearity depends on how well the absorbance bands are matched with the LED emission spectra. If the molar absorptivity, ε , varies only slightly across the emission band of the LED, then the deviation from linearity is usually small. For this reason the two spectra should be compared carefully when choosing an LED for a given application. A further discussion of this effect can be found in Macka et al. [31] as well as in two of our earlier publications [16,32].

Absorbance measurements are best implemented with a log ratio amplifier as illustrated in Fig. 8. These amplifiers give an output voltage (V_O) that is logarithmically related to the ratio of two input currents (i_1 and i_2):

$$V_O \approx \log \frac{i_1}{i_2} \quad (4)$$

As shown in the figure, two photodiodes, one of which provides the reference signal, can be connected directly in the photocurrent mode to the log ratio amplifier. The output voltage represents directly Absorbance, A , i.e. typically 1 V equals an absorbance of 1 (or 1 absorbance unit, 1 AU). The referenced approach will

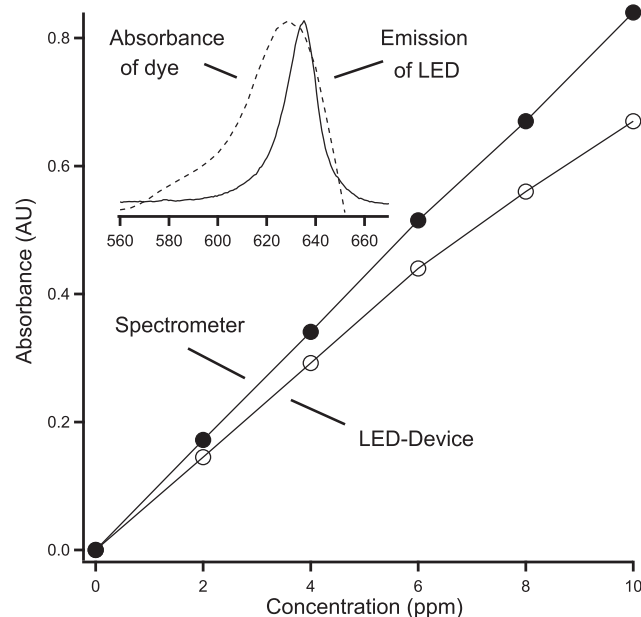


Fig. 7. Absorbances measured for methyl green measured with a device based on a red LED and a photodiode against absorbances measured with a conventional spectrometer. The non-linearity is a violation of Lambert–Beer's law, occurs despite the peaks of absorbance and emission being very close ($\lambda_{\max} = 629$ nm and 635 nm), and is due mainly to the changing absorptivity of the dye across the emission spectrum of the LED.

significantly reduce drifts such as those caused by temperature changes, but can, of course, not completely eliminate them. Residual drifts between 0.3 mAU h^{-1} and 5 mAU h^{-1} were reported, for example, by Bomastyk et al. for such an arrangement [32]. Absorbances down to the low μAU level can be measured with a log ratio amplifier and short term noise levels as low as about $10 \mu\text{AU}$ are readily possible [32]. Integrated logarithmic amplifiers used to be fairly expensive, and thus not suitable for the low-cost applications typical for many of the reported analytical uses of LEDs, but they are now available for about 10–20 US\$ when sourced through catalogue distributors. Log amps suitable for absorbance measurements are presently available from at least three suppliers (Texas Instruments, Analog Devices and Maxim Integrated). Note, that for absorbance measurements there is not much to be gained by using LEDs with intensities higher than average (due to the ratio measurement). Shot noise would ultimately be obtained for low light levels, but even the low intensities of current deep-UV-LEDs are far from this limit [33].

For less demanding applications the reference photodiode can be omitted, but the log amplifier is still useful for carrying out the logarithmic conversion demanded by Lambert–Beer's law. Again for unexacting tasks a logarithmic signal that is inversely

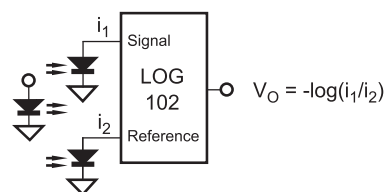


Fig. 8. The use of a log-ratio amplifier to obtain a voltage signal that directly represents absorbance.

proportional to absorbance may also be obtained by using a photodiode in the photovoltaic mode. As has been shown by Bui and Hauser [21] for a LED–photodiode pair this measurement may be carried out directly with a multimeter, instead of a circuitry based on an operational amplifier, as the load by such a standard voltmeter was found tolerable. A variation of this approach is suggested here. Most microcontrollers, such as the AVR-series from Atmel employed on the open source Arduino electronics platform (www.arduino.cc) include an analogue-to-digital convertor (ADC). It is possible to directly connect a photodiode to such an input in order to carry out measurements in the photovoltaic mode, an approach which to our knowledge has not been reported before. The results of such measurements are shown in Fig. 9.

For many of the devices intended for absorption measurements reported in the literature a logarithmic conversion has not been implemented and simply a measurement of light intensity has been used. This parameter is proportional to Transmittance, T , which does not have a linear relationship to concentration. It is, of course, to some extent possible to work with non-linear calibration curves, and furthermore, for a limited concentration range (corresponding to small maximal absorbances) the deviation from linearity may be tolerable (see also the discussion by Dasgupta et al. [8]).

3. Applications

3.1. Molecular absorption spectrometry

3.1.1. Batch measurements

The photometric determination of metal ions following their complexation with colour forming reagents is versatile and widely used and several common anions can also be determined with this long established technique. The absorption bands of molecules, and of the metal complexes, are generally broad and therefore can be well matched to the emission bands of LEDs. However, surprisingly few of the original publications on molecular absorption measurements with LEDs as light source deal with the construction of photometers for batchwise measurements, *i.e.* with replacements of standard photometers. A reason for this must be the appeal of LEDs for miniaturization

which led to a focus especially on detector cells for flow-injection analysis. However, LED based photometers for the measurement of individual samples are still useful and the first publication on the use of LEDs in analytical chemistry by Flaschka et al. indeed described such a device [4]. Real applications were not reported but the cell was evaluated with solutions of a Cu(II)–aquo complex using a red LED. An early report by Imasaka et al. [34] on batchwise measurements concerned the sensitive determination of phosphate with the molybdenum blue method. A detection limit in the sub-ppb level was achieved. LED-based photometers can be compact and battery operated and such field portable instruments are now commercially available from various suppliers (for example from Hach-Lange, Hanna Instruments, Windaus Labor-technik, WTW Wissenschaftlich-Technische Werkstätten, Chemetrics, *etc.*). Typical applications are the determination of chlorine or ozone in swimming pools, or the determination of ammonium or phosphate in waste water treatment plants or in environmental samples. LEDs have also been adopted as light sources into commercial benchtop robotic analyzers for the microtiter format (*e.g.* from Tecan, Biorad, Biochrom, Labexim Products, *etc.*) with their main applications in the clinical field or in life science research, or in a clinical analyser based on a rotary sample tray (Eurolyser). In these routine applications wavelength flexibility is not required.

The fixed wavelength of LEDs can indeed be seen as a disadvantage, and to address this limitation a number of authors have reported switchable multi-wavelength photometers based on dual- or triple-colour LEDs, *i.e.* components which contain two or three emitting chips of different colours, or photometers which combine several standard LEDs [14–16]. The challenge to efficiently combine the light from a number of LEDs into one spot can be solved by employing fibre bundles or fibre splitters. Such LED-array photometers have been reported for the simultaneous determination of binary mixtures of analytes with overlapping spectra in combination with multivariate calibration [15,35]. Another approach to obtain wavelength flexibility from LEDs is to use white LEDs in combination with a wavelength selective device. Veras et al. combined such an LED with a compact disc (CD) as an inexpensive wavelength dispersive device, in order to construct a simple spectrophotometer for molecular absorption spectroscopy [36]. Piasecki et al. replaced the standard deuterium lamp in a commercial diode array detector for capillary electrophoresis with a white LED [37]. For the LED less noise was present compared to the standard light source, which demonstrates that this arrangement still can be advantageous despite the loss of the wavelength selectivity inherent to the light source.

A very special but well established application of absorbance measurements with LEDs is pulse oximetry, in which the oxygen content of blood is measured with a red LED at 600 nm via the colouration of haemoglobin. A probe is clipped directly onto a finger or an earlobe for direct non-invasive *in-vivo* measurement. To account for such variations as the length of the light path and light scattering, a second measurement is made with an IR-LED at 950 nm and the two measurements are ratioed [38].

3.1.2. Titrations

Another early reported analytical application of LEDs was in probes for end-point detection in titrations [39]. This approach has also been adopted commercially and is implemented in state-of-the-art automatic titrators (Metrohm, Mettler-Toledo). One of the advantages of the optical end-point detection in titrations, instead of employing electrochemical sensors, is the possibility of automation of older methods which had been validated for use with indicator dyes.

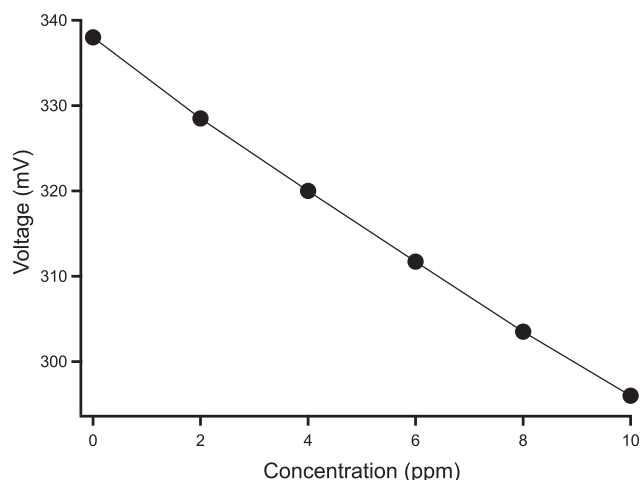


Fig. 9. Voltages on a photodiode operated in the photovoltaic mode measured directly using the analogue-to-digital convertor (ADC) of the microcontroller (ATmega328 from Atmel) on the Arduino Uno platform. Thymol blue in 0.1 M NaOH. LED: $\lambda_{\text{max}} = 595 \text{ nm}$.

3.1.3. Flow systems

An important field of application of absorbance measurements with LED based devices is detection in analytical flow-through systems. Some of the earliest applications reported concerned detectors for flow-injection analysis (FIA), where a reagent is added to produce a coloured product, and these have been reviewed by Trojanowicz et al. [40]. For such cells attention has to be paid to potential refractive index effects (changes of intensity due to changes in the refractive index of the solution when the sample passes through the cell). Such interferences may be minimized by optimizing the cell geometry [8]. It is also possible to use a referenced approach in which a second wavelength at which the analyte does not absorb is employed for, at least, partial correction of refractive index effects or turbidity [41]. The use of LEDs lends itself well to the construction of miniature devices, and even flow through cells constructed by drilling appropriate holes into the body of an LED have been reported (see for example Dasgupta et al. [8]). Flow-through process analysers with LED based absorbance detectors are now commercially available (e.g. from Metrohm). Process monitoring without adding reagents is also possible if the stream shows an absorbance in dependence of the analyte concentration at a wavelength accessible with LEDs [42].

3.1.4. Chromatography

LED based detectors have also been reported for column chromatography. Early reports were based on visible LEDs. Schmidt and Scott in 1984 reported a detector based on a green LED for the determination of heavy metal ions as their complexes with 4-(2-pyridylazo) resorcinol (PAR) [43], a method which was later also used with a PEDD device [44]. Berthod et al. in 1990 described an indirect HPLC detector which was based on the displacement of methylene blue in the eluent by the analytes [45]. Standard photometric detection in HPLC is based on UV-light below 300 nm as most analytes are not coloured and absorb light only in the lower UV-range, and therefore only the commercial introduction of deep-UV-LEDs a few years ago really opened up the field. Detectors based on 255 nm and 280 nm LEDs were reported by Hauser and co-workers [7,32,33]. A detector for conventional HPLC showed detection limits of 0.4 mAU, excellent linearity (correlation coefficients of 0.9999) and reproducibilities of between 0.1 and 0.2%; performance data which is comparable to that of a commercial diode-array detector with a significantly higher purchase price [32]. A photo of a prototype detector for narrow bore separation columns is shown in Fig. 10 and a chromatogram acquired with this device is given in Fig. 11. Note, that for this application special visible light blind photodiodes were employed, in order to suppress a background signal due to secondary emission at longer wavelengths present for the UV-LEDs. Kraiczek et al. recently described an HPLC detector which featured an array of UV-LEDs for flexibility of the wavelength [14].

3.1.5. Absorption detection in capillaries/capillary electrophoresis

Absorption measurements in capillaries are a challenge for three reasons. First of all, in the usual transversal measurement, the optical pathlengths are very short (the internal diameters are 100 μm or less in capillary electrophoresis), which according to Lambert–Beer's law imparts a low sensitivity. It also means that the light source should be highly stable to achieve good precision and low limits of detection. Secondly, the light path needs to be restricted to the narrow internal diameter of the capillary in order to avoid stray light. A carefully produced and aligned optical slit is needed. Thirdly, the assembly has to be very stable mechanically as slight changes in the alignment can strongly influence the intensity of light falling on the detector. On the other hand, the inherent stability, high intensity, and small size of LEDs are an advantage for such applications and a number of LED based absorption detectors

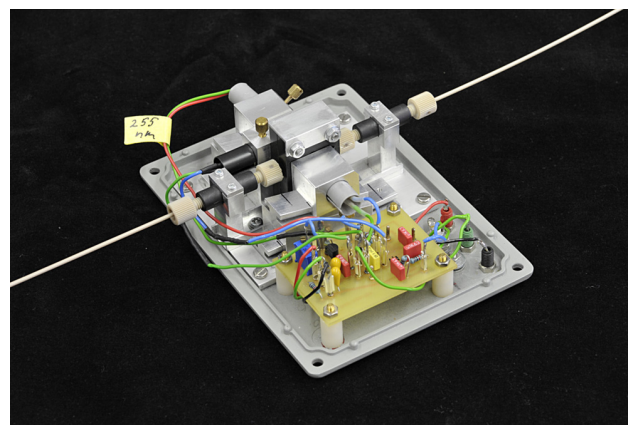


Fig. 10. Detector for narrow bore HPLC based on a deep-UV-LED.

for capillary electrophoresis (CE) and other flow methods employing capillaries have been developed. The first report on the use of such a cell in CE by Tong and Yeung in 1995 described the employment of yellow and red LEDs and a cell that included focusing optics (a camera lens and two ball lenses) [46]. Direct absorption detection, as well as indirect detection were demonstrated. The latter refers to the use of a charged dye in the separation buffer which is displaced by the analyte ions leading to negative going peaks [46]. This approach is commonly used in CE, also when employing conventional absorption detectors, when ions which do not absorb light are to be detected. This early report was followed in 1996 by a study by Macka et al. in which the mercury vapour lamp in a conventional CE detector was replaced by an LED and the superior performance of the solid state light source was demonstrated [31]. Boring and Dasgupta in 1997 described a compact purpose made cell that did not require any focussing optics [47]. A different arrangement, which was based on fibre optic cables butted perpendicularly to the separation capillary, was also reported in 1997 [48]. The latter approach dispenses with the need of placing an optical aperture in front of the capillary as the size of the fibre core can be made to match the internal diameter of the separation capillary.

To improve the sensitivity of absorbance measurements in capillaries Mishra and Dasgupta in 2007 reported a multi-reflection cell [49]. Another approach to increasing the sensitivity for optical detection in capillaries is by passing the light axially along the capillary, instead of the usual transversal arrangement. This is possible by employing capillaries which are made from a

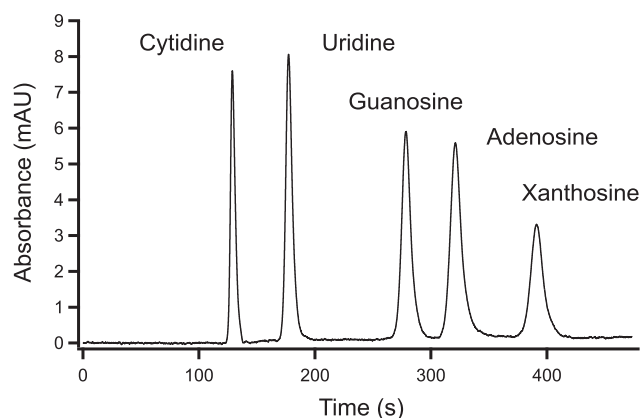


Fig. 11. Chromatogram for nucleosides at 25 μM acquired with the detector shown in Fig. 10.

low refractive index material (Teflon AF), or which are internally coated with such a material, in order to obtain a liquid-core waveguide. Wada et al. [50] reported such a set-up for a study in capillary electrophoresis in which the light could be guided along its entire length, but it is difficult to see how this could be implemented for the usual end-of-capillary detection in electrophoresis. However, other analytical applications of liquid-core waveguides in capillaries have been described, some of them involving LEDs as light sources, and these have been reviewed by Dallas and Dasgupta [51].

Macka and co-workers have also remained active in the field of LED based absorption detectors for CE and over the years have demonstrated a range of applications [52–61]. This included, for example, a study of the use of a near-UV-LED (380 nm) with chromate, which is frequently used as displacement dye in the indirect detection of inorganic anions [62]. The developments of LED based detectors in CE up to 2009 have been reviewed by Xiao et al. [63,64].

As for HPLC, in capillary electrophoresis the deep UV range is the most important wavelength region. A detector employing an LED with an emission band at 255 nm which was based on a modified cell from a conventional CE detector, was reported by Krčmová et al. in 2009 [17]. Due to the relatively low intensity of the early deep-UV device, and the loss of light at the narrow cell aperture, a photomultiplier tube had to be employed for the measurement of the transmitted light intensity. A similar detector based on a 255 nm LED was also reported by Rudaz and co-workers [65,66], but unfortunately very little detail, other than that it employed a deep-UV-LED as light source was given.

LED based detectors for CE are, to our knowledge, presently not commercially available, but a suitable capillary cell which may be used with LEDs is on sale (Ocean Optics). This employs butted fibre optic cables and is based on a commercial cross-type tubing coupler.

3.1.6. Gas phase

The determination of small analytes by absorption measurement in the gas phase is a well established technique. A prominent species is carbon dioxide (CO_2), but many others, such as methane (CH_4) and other hydrocarbons, ozone (O_3), NO_x compounds, sulphur dioxide (SO_2), hydrogen sulfide (H_2S), ammonia (NH_3), or carbon monoxide (CO) are also of interest. For a review on optical gas sensing see for example [67]. These analytes show groups of very narrow absorption lines in the mid-IR range (2.5–14 μm) due to the vibrational and rotational modes of the molecules. Spectral matching to a single line requires the narrow emission line of a laser, and indeed powerful laser based techniques are available, which allow sensitive determination and may even be used for the distinction between molecules composed of different isotopes. For the construction of simple instruments or sensors, broadband sources, such as incandescent lamps, are employed. Typical applications are air monitoring in industrial settings or in mining. These give good results for not too challenging limits of detection despite the not perfect spectral match. Different cell arrangements are used and these usually include an optical filter to limit the wavelength range and often relatively long pathlengths to achieve good sensitivity. Another difficulty is the presence of a background radiation in the IR-range, and for this reason the light source is generally intensity-modulated to allow discrimination via synchronous detection [68]. The replacement of thermal sources with LEDs is advantageous not only due to their inherent wavelength selectivity but also because they can be electronically modulated. The presence of the background radiation also makes the use of wavelength selective detectors sensible for this wavelength range in order to suppress some of the unwanted baseline signal, and for example Matveev

have described the use of specially designed spectrally matched pairs of LEDs and wavelength restricted photodiodes for optical gas sensing [68].

Probably the earliest report on a gas sensor based on a direct absorbance measurement and employing an IR-LED was published by Johnston in 1992 [69]. Two devices for monitoring carbon dioxide at 4.25 μm and hydrocarbons in the range from 3.3–3.4 μm were described. Since then, a variety of photometric absorbance sensors employing mid-IR LEDs have been reported, for example for the detection of CH_4 [70–72], CO and CO_2 [73], and water vapour [73]. The commercial adoption of the mid-IR LEDs for this purpose has been relatively slow, possibly because these devices are not mass-produced for other applications, but some products are commercially available (e.g. from LED Microsensor NT, Gas Sensing Solutions, and Mipex). An added advantage of these devices is the lower power consumption compared to the ones based on thermal radiation sources, so that this approach is of particular interest for portable gas detectors.

Photometric gas sensing with LEDs is mainly the domain of IR-LEDs, but some fruitful spectral matches outside the mid-IR have also been reported. Fowles and Wayne reported already in 1981 a photometric detector for ozone employing a yellow–orange LED as light source and a silicon photodiode as light sensor [74]. It was reported that the detection limit in the ppm range was achieved with a relatively short optical pathlength for gases of 10 mm. The introduction of UV-LEDs opened up some further possibilities. Degner et al. [6], Kalnajs and Avallone [75], as well as Aoyagi et al. [76] reported ozone sensors based on deep-UV-LEDs with 280 or 255 nm emission bands and achieved detection limits below 100 ppb. Degner et al. also reported the determination of SO_2 and NO_2 in exhaust gases from internal combustion engines via their absorbance bands at 280 nm and 400 nm respectively using LEDs [77]. Schorsch et al. reported the use of a blue and a UV-LED of 370 nm for the detection of OH and CH radicals in flames [78].

3.2. Fluorescence

LEDs may also be employed as excitation sources in molecular fluorescence spectrometry. For this application the shorter wavelengths are more important as most fluorescent compounds require blue or ultraviolet light for excitation. In contrast to molecular absorbance measurements for fluorescence measurements LEDs of the highest intensities are desirable as the signal, and therefore the detection limit, is directly proportional to the intensity of the excitation source. Generally, much lower detection limits are achieved in fluorescence than in absorption measurements. The intensities of LEDs tend not to be as high as those of lasers, but this is, at least partially, compensated by a higher stability. The suitability of LEDs for fluorescence measurements is illustrated in Fig. 12.

In a very early report in 1975 an instrument based on a red LED for the measurement of the fluorescence of chlorophyll directly in leaves was described [79]. Another early report on the use of an LED for a fluorescence measurement in 1986 described a fibre optic end-point detection system for acid–base titrations employing a coumarin indicator dye [5]. Smith et al. pursued more standard fluorimetry and presented a simple fluorimeter with a green LED and a photodiode employing a conventional cuvette [80]. Their study was focussed on the improved noise and stability that could be obtained with LEDs compared to conventional light sources.

A prominent application over the years has been fluorescence detection in capillary electrophoresis. The first publication by Bruno et al. [81] demonstrated the simple detection of rhodamine B standard excited with a green LED, but the method is particularly useful for the determination of fluorescently labelled biochemical species, such as amino acids, peptides, proteins, DNA fragments,

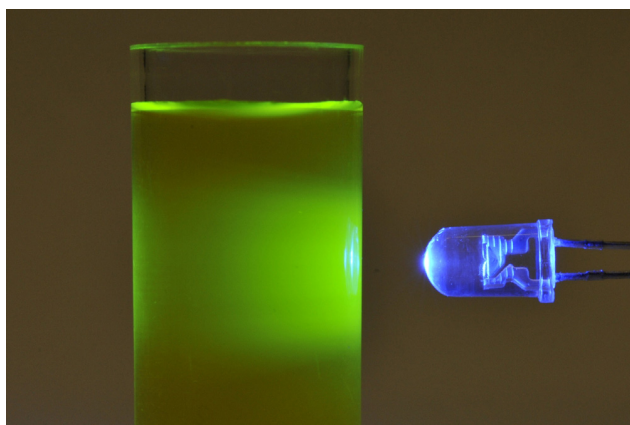


Fig. 12. Excitation of fluorescence from fluorescein using a blue LED.

catecholamines, and others. The developments up to 2009 have been summarized by Xiao et al. in their reviews on the use of LEDs in capillary electrophoresis [63,64]. This work has mostly been carried out with blue LEDs which are available with high intensity and match the excitation wavelengths of common fluorescent labels, such as fluorescein.

In fundamental studies of fluorescence frequently lifetime measurements are made using pulsed light sources. LEDs are again well suited for this task as they can be electronically modulated reaching into the nanosecond domain. This approach was first demonstrated by Araki and Misawa in 1995 with a blue LED using a special pulse shaping circuitry and a ultrafast digitizing oscilloscope [82]. Harms et al. soon after demonstrated a more affordable set-up based on a commercial lock-in amplifier [83]. An example of an application for such measurements is the detection of polycyclic aromatic hydrocarbons (PAHs) in environmental samples [84]. Recently the time domain technology has also been implemented in a very compact portable format [85].

The fact that in fluorescence the sensitivity is directly proportional to the intensity of the excitation source has been exploited for single molecule detection using lasers. It has also been shown more recently by different authors that single molecule detection can also be achieved with high intensity LEDs [86–88]. Also possible is gas-phase fluorescence with LED sources. Sadanga et al. have described the determination of atmospheric nitrogen dioxide with a blue light-emitting diode via its fluorescence [89]. The use of LEDs for the excitation of fluorescence has not been limited to molecules, atomic fluorescence of alkali and alkaline earth metals in the inductively coupled plasma with these sources has also been reported [90].

A very useful application of LEDs is as light sources in fluorescence microscopy for the investigation of stained biological samples. According to Marzouk et al. in such applications LEDs are a replacement for conventional mercury lamps with improved spectral properties and higher intensities, which eliminates the need for a darkened room [91]. This provides, for example, easier access to sputum tests for tuberculosis in developing countries [92]. Such microscopes are now commercially available as are LED based instruments for time domain fluorescence studies (e.g. from Photon System Instruments), special instruments for algae or chlorophyll determination (e.g. from Qubit), handheld portable instruments for environmental testing such as the determination of optical brighteners in waste water or cyanobacteria in fresh water (e.g. from Turner Designs), portable instruments for chlorophyll measurements in leaves (e.g. from Walz Mess- und Regeltechnik),

instruments for total DNA quantification (e.g. from Biotium) or for determination of DNA fragments following PCR (e.g. from Biosan) and others.

3.3. Membrane based sensors

Optical chemical sensors (“optodes” or “optrodes”) generally are based on membranes which interact specifically with an analyte and in the process change an optical property. Fluorescence measurements are more easily implemented than absorbance measurements as both, the excitation and the measurement of the signal, cannot be carried out from the back of the membrane which is not exposed to the sample. An early report on an LED-based all solid-state instrument for use with optical sensors was presented by Guthrie et al. 1988 [93]. Among the many examples reported in the literature the most prominent application is the determination of dissolved oxygen via fluorescence quenching of, for example, ruthenium complexes [94] and such sensors are now commercially available from different suppliers. The end of a fibre optic probe, which was used in our laboratory, is shown in Fig. 13 [29]. It was employed with fluorescent membranes, for example for the determination of nitrate. The probe consisted of a bifurcated fibre bundle, one half of the bundle was used to bring light from the LED to the sensing membrane, while the other half served to bring light back to the detector. Lock-in amplification allowed the distinction from ambient light. Taib and Narayanaswamy in 1995 [95] reviewed fibre optic sensors employing solid state instrumentation, which largely implies LEDs or laser diodes as light sources.

3.4. Microfluidic devices

The small volumes of microfluidic devices pose the same challenges as capillaries. As the optical path lengths tend to be even shorter, absorbance measurements are relatively seldom used in favour of fluorescence measurements. Götz and Karst in 2007 wrote a review on optical detection methods for microchip separations which includes the LED based methods [96]. A nice example is the compact microchip electrophoresis analyzer for creatinine in urine following labelling with fluorescein isothiocyanate described by Wang et al. [97]. The compact instrument was based on a blue LED and a photodiode for quantification.

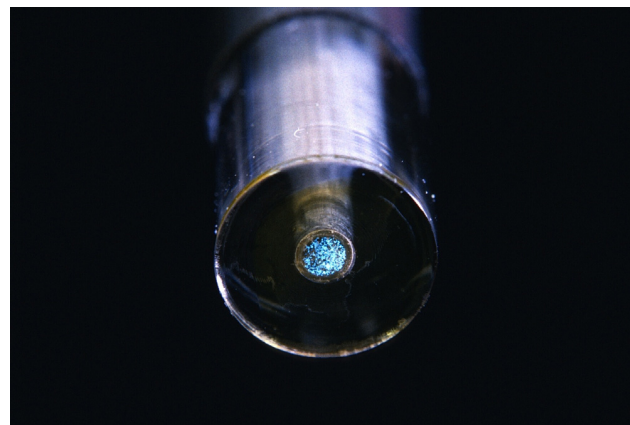


Fig. 13. The end of a bifurcated fibre optic probe for use with fluorescent sensor membranes.

3.5. Photoacoustics

Photoacoustic spectroscopy is a thermo-optic method in which the absorption of light is measured indirectly via the heat waves created when the sample is irradiated with intensity modulated light. The heat waves translate into pressure waves, i.e. the propagation of sound according to the frequency at which the light source is modulated. The sound can then be picked up with a microphone or a piezoelectric transducer. As it is not a differential measurement, high intensity light sources lead to low limits of detection, a feature shared with fluorimetry. Lasers are often also used for this reason, but the introduction of high intensity LEDs brought about some examples of instruments based on these as well. Lay-Ekuakille et al. described a gas monitor based on mid-IR LEDs (4–4.5 μm) for applications in a hospital such as the determination of the anaesthetics sevoflurane and nitrous oxide (N_2O) [98]. Water vapour may be determined with a near-IR LED (1450 nm) [99]. NO_2 in air can be determined with a blue LED (about 460 nm) also by photoacoustics [100–102], and ozone in air is accessible with a deep-UV-LED (285 nm) [103]. Rabasovic et al. reported a low-cost photoacoustic instrument for solid samples employing a white LED [104].

3.6. Other applications

An unusual spectrometric application of LEDs is their use in Raman spectroscopy, which was reported by Adami and Kiefer [105]. Note that in order to achieve good spectral resolution, a further narrowing of the emission band of the LED was necessary, which was achieved by placing an interference filter in front of the LED.

It was recognised already in 1978 by Betteridge et al. that LED based transducers can be employed for refractive index measurements on liquids in flow through systems [106] and Pawliszyn studied this effect further in 1987 [107]. This application has now also been implemented commercially. Refractive index detectors for HPLC with LEDs as light sources are produced (e.g. by Waters), and benchtop instruments for the batchwise determination of the refractive index of solutions and solvents employing LEDs are also available (e.g. from Schmidt + Haensch).

Turbidimetry and nephelometry may also be carried out with LEDs. Schnable et al. [108], for example, in 1998 described a portable nephelometer featuring LEDs of different wavelengths. dos Santos et al. reported an LED-based instrument for concurrent turbidimetric and nephelometric measurements of pharmaceutical formulations [109]. Strzelak and Koncki described a flow-through cell for turbidimetry/nephelometry of urine proteins [110]. Benchtop and portable turbidimeters (employing near-IR LEDs) are now available commercially (e.g. from Hanna and Hach).

4. Conclusions

The development of analytical applications of light-emitting diodes has been very successful as evidenced by the diversity of reported methods. There is a clear trend to the replacement of conventional light sources in commercial instruments for applications where wavelength flexibility is not required. It has also been a great success story for academic research in analytical chemistry, indeed an endorsement, as many of the approaches first published in the scientific literature have later become implemented in routinely used commercial instrumentation. Further progress can be expected, in particular with regard to the important deep-UV range where most organic molecules show an absorbance.

Acknowledgments

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