Photoacoustic Spectroscopy and Its Applications – A Tutorial Review

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Abstract

Based on photoacoustic effect, photoacoustic spectroscopy is an unusual form of spectroscopy which uses both light and sound and is based on the absorption of electromagnetic radiation by analyte molecule. The absorbed energy is measured by detecting pressure fluctuations in the form of sound waves or shock pulses. A photoacoustic spectrum consists of a plot of the intensity of the acoustic signal detected by a microphone or a piezoelectric detector, against the excitation wavelength or another quantity related to the photon energy of the modulated excitation. It is a valuable source for data acquisition relating to absorption and material science studies.

1. Introduction

Photoacoustic spectroscopy (PAS) is based on the absorption of electromagnetic radiation by analyte molecules. Non-radiative relaxation processes (such as collisions with other molecules) lead to local warming of the sample matrix. Pressure fluctuations are then generated by thermal expansion, which can be detected in the form of acoustic or ultrasonic waves (Fig. 1). In other words, the transformation of an optical event to an acoustic one takes place in photoacoustic spectroscopy [1]. A fraction of the radiation falling upon the sample is absorbed and results in excitation, the type of which being dependent upon the energy of the incident radiation. Non-radiative de-excitation (relaxation) processes which normally occur give rise to the generation of thermal energy within the sample. If the incident radiation is modulated then the generation of thermal energy within the sample will also be periodic and a thermal wave/pressure wave will be produced having the same frequency as this modulation. Energy is transferred by the thermal wave/pressure wave towards the sample boundary, where a periodic temperature change is generated. The periodic variation in the temperature at the surface of the sample results in the generation of an acoustic wave in the gas immediately adjacent and this wave propagates through the volume of the gas to the detector (microphone, piezoelectric transducers or optical method) where a signal is produced. This detector or microphone signal, when plotted as a function of wavelength, will give a spectrum proportional to the absorption spectrum of the sample. Therefore, the photoacoustic signal is a function of two types of processes occurring in the sample: the absorption of electromagnetic radiation specified by the absorption coefficient β and the thermal propagation in the sample specified by the thermal diffusivity, x [2].
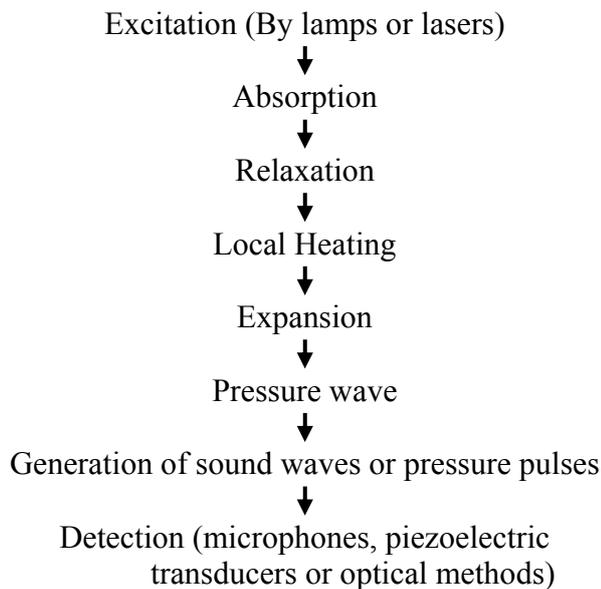
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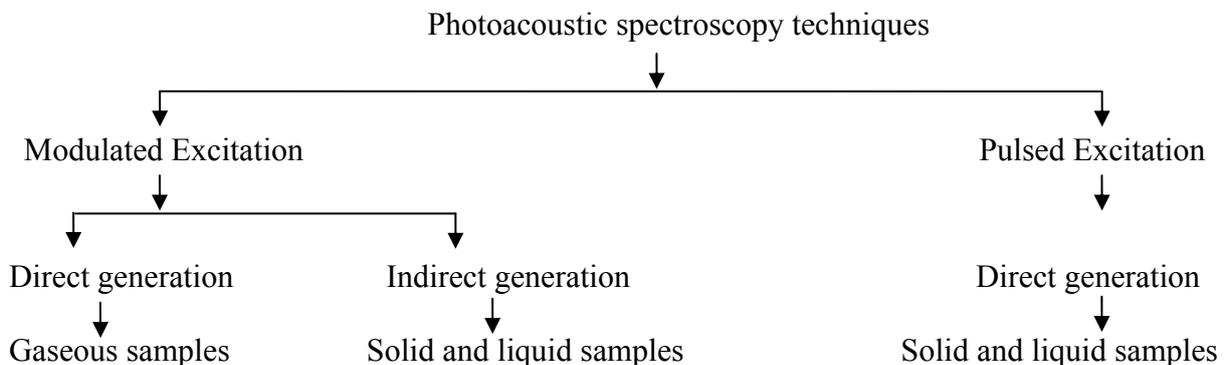 **E-mail:** deepvision85@gmail.com

The photoacoustic effect was discovered by A.G. Bell in 1880 [3]. He found that thin discs emit sound when exposed to a rapidly interrupted beam of sunlight [4]. By placing different absorbing substances in contact with the ear using a hearing tube, he was able to detect absorption in both the visible and the invisible regions of the solar spectrum. This spectrophone was used in his experiments on wireless transmission of sound. After additional experiments by Tyndall and Rontgen, and some initial analytical applications in the 1930 and 1940, interest in the photoacoustic effect declined over the following decades. The first applications of the effect to trace gas monitoring were reported in the late 1960. Important steps leading to this rediscovery of the effect for analytical purposes were the invention of the laser as an intense light source, the development of highly sensitive sound detectors (such as condenser microphones and piezoelectric transducers), and the first comprehensive theoretical description of the photoacoustic effect in solids by Rosencwaig and Gersho: the so-called RG theory [5].

Excitation (By lamps or lasers)

↓

Absorption

↓

Relaxation

↓

Local Heating

↓

Expansion

↓

Pressure wave

↓

Generation of sound waves or pressure pulses

↓

Detection (microphones, piezoelectric
transducers or optical methods)

Fig. 1. Principle of excitation, signal generation and detection in a photoacoustic experiment.

2. Techniques involved in photoacoustic spectroscopy

In this section, various schemes for the excitation, generation, and detection of Photoacoustic signals are presented.

Photoacoustic spectroscopy techniques

Modulated Excitation   Pulsed Excitation

Direct generation   Indirect generation   Direct generation

Gaseous samples   Solid and liquid samples   Solid and liquid samples

2.1. Excitation [1, 6-13]

As mentioned earlier, the Photoacoustic effect is based on the sample heating produced by optical absorption. In order to generate acoustic waves, which can be detected by pressure sensitive transducers, periodic heating and cooling of the sample is necessary to generate pressure fluctuations. In principle, there are two ways to realize Photoacoustic pressure fluctuations: modulated and pulsed excitation.

2.1.1. Modulated excitation

In modulated excitation schemes, radiation sources are employed whose intensity fluctuates periodically in the form of a square or a sine wave, resulting in a 50% duty cycle. This can be realized for example by the mechanical chopping of a light source. A way to overcome the 50% duty cycle is to modulate the phase instead of the amplitude of the emitted radiation. Whereas chopped or modulated lamps or IR sources from commercial spectrometers are used for the determination of UV/Vis or IR absorption spectra of opaque solids, modulated continuous wave (cw) lasers are the most common sources for Photoacostic gas phase analysis. The modulation frequencies usually range from a few Hz up to several kHz. The resulting pressure fluctuations generate sound waves in the audible range, which can be detected by microphones in the gas phase. Data analysis is performed in the frequency domain. Therefore, lock-in amplifiers are used for signal recording, which allows the analysis of both the amplitude and the phase of the sound wave. Since the acoustic propagation during the relatively long illumination period is much larger than the dimensions of the sample in most cases, boundary conditions have to be taken into account. This means that eigenmodes of the PA cell play an important role. This fact can be utilized for signal enhancement by acoustic resonance. Thus, acoustic resonance curves must be considered in PA cell design. In solid samples, tuning of the modulation frequency allows depthresolved investigations.

2.1.2. Pulsed excitation

In pulsed PAS, laser pulses with durations in the nanosecond range are usually employed for excitation. Since the repetition rates are in the range of a few Hz, the result is a short illumination followed by a much longer dark period: a low duty cycle. This leads to a fast and adiabatic thermal expansion of the sample medium resulting in a short shock pulse. Data analysis in this case is performed in the time domain. Therefore, the signal is recorded by oscilloscopes, boxcar systems, or fast A/D converters. Transformation of the signal pulse into the frequency domain results in a wide spectrum of acoustic frequencies up to the ultrasonic range. Thus, laser beams modulated in the form of a sine wave excite one single acoustic frequency, whereas short laser pulses are broadband acoustic sources. In solid samples, analysis of the time delay between laser pulse and pressure detection allows depth profiling.

2.2. Signal generation [1, 6, 14-19]

Induction of an acoustic wave by modulated or pulsed irradiation inside a gaseous, liquid or solid sample is termed as direct Photoacostic generation. Here, detection takes place inside or at an interface of the sample. In indirect Photoacostic generation, heat is generated by modulated illumination inside a solid or liquid sample and transported to an interface. Subsequently, sound waves are generated and detected in the gas phase adjacent to the sample.

2.2.1. Direct PA generation in gases

Chopped continuous wave lasers or modulated laser diodes are employed for the modulated excitation of PA signals in gases. The modulated laser beam irradiates the gaseous sample inside a (usually cylindrical) PA cell, and sound waves with an acoustic frequency defined by the modulation frequency of the laser can be detected using microphones. The signal amplitude can be described by, $p = FW_0\mu_0$, where W_0 is the incident radiation power and *a* is the absorption coefficient of the sample. The proportionality factor *F* is termed the cell constant. In the so-called non-resonant mode, the modulation frequency is much lower than the first acoustic resonance frequency of the photoacoustic cell. In this case, the wavelength of the generated acoustic wave is larger than the cell dimensions. Thus, the generation of standing acoustic waves is not possible. The cell constant *F* is, F= G (γ-1) L/ωV. Here, *G* is a geometric factor of the order of one, γ is the adiabatic coefficient of the gas, *L* and *V* are the length and the volume of the cell, and $\omega = 2\pi v$ the modulation frequency. The photoacoustic signal is indirectly proportional to the modulation frequency and the cross-section *V/L* of the cell. Thus, the signal increases with decreasing cell dimensions and modulation frequency. As the noise increases with a decrease in these parameters, there is a maximum in the *S/N* ratio for a certain combination of cell size and modulation frequency.

With increasing modulation frequency, at a certain point the acoustic wavelength reaches the cell dimensions, and the resonant eigenmodes of the cell can be excited, leading to an amplification of the signal. Resonance properties mainly depend upon the geometry and size of the cavity. The modulation frequency is tuned to one of the eigen frequencies of the cell and thus the signal is amplified by a quality factor Q_i resulting in a cell constant F_{res} F_{res}=QC$_i$(γ-1)/ ω_iV, where C_i denotes a factor that depends on the positions of the laser beam and the microphone relative to the pressure distribution in the cell. The index *i* indicates that *Q* and *C* are parameters describing the *i* th eigenmode of the cell. The quality factor can be described as the ratio between the accumulated energy in the resonator and the energy loss per cycle. In resonant cells with high quality factors, the resonance profile becomes narrow and small drifts of modulation frequency or speed of sound in the gas mixture cause strong changes in *Q*. Therefore, moderate *Q*-factors are preferred in many applications. As mentioned, pulsed excitation leads to broadband generation of various acoustic frequencies. Thus, in gas phase analysis by pulsed laser PAS, all eigenmodes of the cell can be excited simultaneously. The cell design is usually optimized to the excitation of one selected eigenmode, which is well separated from the neighboring ones. In this case, each laser pulse generates an exponentially decaying sine wave whose amplitude can be described by, p=C (γ-1)E$_0\mu_a$/V. Here, E_0 is the laser pulse energy. Note that the PA signal amplitude does not depend on *Q*-factor, modulation frequency or repetition rate. Thus, the signal does not depend on the speed of sound in the cell. In this case, the cell constant depends only on the geometrical properties of the PA cell. If the signal is dominated by one eigenmode and the contributions of other eigenmodes can be neglected, cell constants can be calculated that are in good agreement with experimental results. Pulsed laser PAS can therefore be applied to absolute absorption measurements in gases.

2.2.2. Direct PA generation in liquids and solids

In condensed matter, short laser pulses are used for direct PA generation. The short illumination with relatively high peak power leads to an instantaneous adiabatic expansion of the medium, generating pressure pulses that propagate through the sample at the speed of sound. These ultrasonic pulses can be detected directly at a boundary of the sample by piezoelectric transducers or optical methods. As in other PA techniques, the signal amplitude depends linearly on the excitation energy and the absorption coefficient of the sample, and

they can be described by, $P \propto \beta c^2 E_0 \mu_a / C_p$, where β, c and C_p denote the samples thermal expansion coefficient, speed of sound, and heat capacity, respectively. If the signal in pulsed PAS is recorded time-resolved, the time delay t between laser pulse and pressure detection can be determined, which represents the propagation time of the ultrasonic pulse through the sample. Thus, the depth z of an absorbing object inside the sample can be calculated simply as follows $z = c\ t$. This equation corresponds to the principle of ultrasonic tomography. It should be pointed out that in ultrasonic tomography; reflections of pressure waves at acoustic impedance mismatches are detected. Thus, for a signal to occur in ultrasonic tomography, a change of the acoustic impedance Z at an interface is needed, which is defined as $Z = \rho$, where ρ is the density of the sample. In contrast to ultrasonic tomography, PA depth profiling allows the depth resolved detection of changes in optical absorption. The maximum depth that can be investigated by pulsed PAS is limited by the optical penetration depth, $\delta = 1/\mu a$. Since the decay length of acoustic waves is much higher than the optical penetration depth in many solid samples, beyond this limit depth profiling is possible by detecting laser-induced pressure pulses, which are reflected at acoustic impedance mismatches. This technique, which is similar to ultrasonic tomography, can be applied to the nondestructive testing of opaque solid materials, and is termed laser-induced ultrasound (LIU).

Depth resolution of both pulsed PAS and LIU depends mainly on the time resolution of the ultrasonic detector. The depth resolution can be calculated as the product of the temporal resolution of the detector and the speed of sound in the sample. If fast piezoelectric detectors and data recording with temporal resolutions in the nanosecond range are used, depth resolutions in the lower micrometer range can be realized. The maximum sampling depth can reach a few centimeters in weak absorbing and scattering samples. If piezoelectric detector arrays, scanning PA sensors or suitable optical methods for detection are employed, two dimensional and three dimensional imaging are feasible by pulsed PAS.

2.2.3. Indirect PA generation

Analysis of condensed matter by modulated PA excitation and subsequent detection of the directly generated acoustic wave by a microphone is not suitable due to strong acoustic impedance mismatches between solid and gas phase. Thus, an indirect scheme for PA generation is employed. Modulated warming of the sample is induced by modulated excitation. Subsequently, the heat deposited in the sample is transported to the interface of the sample with the adjacent gas phase. This heat transport can be described as thermal wave.

For indirect generation and detection of PA signals, PA cells can be coupled to conventional spectrometers, allowing UV/Vis or IR absorption spectroscopy of opaque solid samples. A relatively common set-up for this purpose is FT-IR–PAS. In this case, a Fourier transform IR spectrometer is used for excitation and PA signals are detected in a PA cell, which is in contact with the sample. In continuous scan FT-IR (CSFT-IR), the modulation frequency can be tuned by changing the interferometer moving mirror velocity. In step scan mode (S^2FT-IR), the excitation beam is modulated mechanically by a chopper for intensity modulation or by jittering the position of one of the interferometer mirrors, resulting in phase modulation.

2.3. Signal detection [1, 6, 17-19]

Sound waves generated directly or indirectly in the gas phase are detected usually by condenser or electret microphones. Detection of sound waves by microphones in condensed matter is typically not suitable. Due to high acoustic impedance mismatches, less than 10^{-4} of the acoustic energy is transferred from a solid sample to the adjacent gas phase. In pulsed excitation of condensed matter, the application of microphones is additionally hampered due

to their restricted bandwidth. Therefore, piezoelectric transducers are employed in many cases for the detection of ultrasonic pulses in liquid and solid samples. Quartz crystals, piezoelectric ceramics such as lead zirconate titanate (PZT), lead metaniobate, and lithium niobate as well as piezoelectric polymer films can be applied to the detection of laser-induced shock pulses.

The most common piezoelectric polymer is polyvinylidene fluoride (PVDF), which is available in different thicknesses ranging from 5 to 100 μm as a transparent film or coated with metals for electrical contact. As the sensitivity of piezoelectric detectors usually increases with their thickness, in general, piezoelectric ceramics are more sensitive than thin polymer films. In piezoceramics, eigenmodes are excited by pressure pulses, leading to acoustic waves that decay exponentially within microseconds to milliseconds. Further pressure pulses reaching the detector within this time are overlaid by the signal from the first pulse. This is not observed when nanosecond pressure pulses are detected by thin piezoelectricpolymer films. Therefore, ceramics are preferable for sensitive quantitative analyses in liquids, whereas piezoelectric polymer films are used, if the temporal pressure distribution is of importance, in pulsed PA depth profiling, for example in the analysis of liquid samples by pulsed PAS, in most cases the so-called forward mode is employed for signal detection. In this mode, excitation and detection are performed on different sides of the sample. In weak absorbers, attenuation of the laser beam inside the sample can be neglected. Thus, the laser builds a cylindrical acoustic source resulting in cylindrical waves, which can be detected perpendicularly to the laser beam. In opaque samples, low optical penetration depth leads to a punctiform acoustic source that generates spherical waves. Here, detection both along the laser beam and perpendicular to it is possible. In pulsed PA analysis of solid samples, the generated pressure pulses are often detected in backward mode, where excitation and detection are performed at the same side of the sample. Since piezoelectric transducers are generally not transparent, illumination through the piezo and detection at the same point are not possible. The PA sensor uses a transparent prism as coupling material for both illumination of the sample and transfer of the acoustic energy to the detector. Another possibility is to illuminate the sample by means of an optical fiber and to detect the pressure pulses by a piezoelectric ring around the fiber.

In addition to conventional acoustic detection based on microphones or piezos, optical detection schemes for pressure waves are described in the literature. Optical microphones are applied in PA gas phase analysis and similar fiber optic sensors are reported for liquid phase analysis. Here, an optical fiber is wrapped onto a PA cell. Pressure changes inside the cell cause refractive index changes in the fiber which lead to phase shifts in the light coupled into the fiber and these can be detected, for example, by interferometry. In this case, the fiber is one arm of an interferometer.

In PA investigations of condensed matter, optical methods can be employed for the detection of refractive index changes at an interface of the sample or measurement of the surface displacement caused by a pressure wave. In the first case, the sample is placed at the base of a transparent prism. A continuous probe laser beam is reflected at the interface between prism and sample. A pressure wave transmitted through this interface will change the refractive index, resulting in variations of the optical reflectance, which can be detected optically. If a time-gated video camera is employed for detection, the pressure waves can be imaged in a two-dimensional and time-resolved fashion.

In the second case, the probe beam is one arm of an interferometer. Pressure waves reaching the interface will cause surface displacements in the nanometer range, which can be detected due to changes in the interference pattern. Such optical methods are advantageous due to their ability to perform non-contact measurements and their improved lateral resolution

compared to piezoelectric detectors. For three-dimensional PA imaging, two dimensional piezoelectric detector arrays have been proposed, but small piezosensors with fine spacing are hard to realize.

In optical detection, lateral resolution is only limited by the diameter of the probe beam, which can be less than 1 μm. Pressure waves generated in liquid and solid samples can be visualized directly by Schlieren photography or dark field imaging. These techniques allow the influence of optical properties and the illuminated volume fraction of the sample on the produced acoustic waveforms to be investigated. Since the sample needs to transmit light beams in order to be able to visualize the pressure waves, these techniques are restricted to transparent and non-scattering samples. To overcome this limitation, an interesting set-up was proposed: an acoustic lens system was used to image the initial transient pressure distribution inside the sample into a water container, where the three-dimensional pressure distribution could be detected by dark field imaging. In this way, absorbing objects inside a scattering matrix could be visualized as stereo images.

3. Instrumentation of photoacoustic spectroscopy

3.1. Radiation Sources

Radiation source can be output from a laser, a monochromator furnishing radiations in UV, IR, or a FT-IR spectrometer (tungsten lamp, carbon arc lamp, high pressure xenon lamp, Nernst glower and lasers.) All radiation must be pulsed at an acoustical frequency 50-1200Hz. PA cell is filled with transparent gas often air or helium and cell volume is kept small, less than $1cm^3$ in order to preserve the strength of the acoustical signal [20-21]. In commercial photoacoustic spectrometers, incoherent sources such as lamps are employed in combinations with filters or interferometers. Devices equipped with a small light bulb, with either a chopper or direct current modulation as modulated source and appropriate filters to avoid absorption interferences with other species, are used as compact gas sensors, e.g. for indoor CO_2 monitoring. However, since the generated photoacoustic signal is proportional to the absorbed (and thus to the incident) radiation power, powerful radiation sources, particularly lasers offering high spectral brightness, are advantageous for achieving high detection sensitivity and selectivity in spectroscopic applications. Diode lasers have so far only rarely been employed in photoacoustic spectroscopy owing to their limited power. This situation may, however, change with the ongoing developments in this field. On the one hand, near infrared diode lasers with sufficient power for PAS are available for monitoring overtones and combination bands of molecular fundamental absorptions. On the other hand, current efforts focus on the implementation of widely tunable narrowband all-solid-state laser devices in the mid-IR region for accessing the (much stronger) fundamental absorptions. Optical parametric oscillators (OPOs) and difference frequency generation (DFG) in nonlinear crystals are certainly of great interest for compact spectrometers. Furthermore, recent developments in quantum cascade lasers look very promising in this respect [6].

3.2. Modulation Schemes

Modulation schemes can be classified into the modulation of the incident radiation and modulation of the sample absorption itself. The first technique includes the most widely used amplitude modulation (AM) of continuous radiation by mechanical choppers, electro-optic or acousto-optic modulators as well the modulation of the source emission itself by current modulation or pulsed excitation. In comparison to amplitude modulation (AM), frequency modulation (FM) or wavelength modulation (WM) of the radiation may improve the detection sensitivity by eliminating the continuum background caused by a wavelength – independent

absorption, e.g. absorption by cell windows, known as window heating. This type of modulation is obviously most effective for absorbers with narrow line width and most easily performed with radiation sources whose wavelength can rapidly be tuned with a few wave numbers. Pulsed excitation is often applied for liquids but is also of interest for gaseous samples because it permits time gating and the excitation of acoustic resonances [6-8].
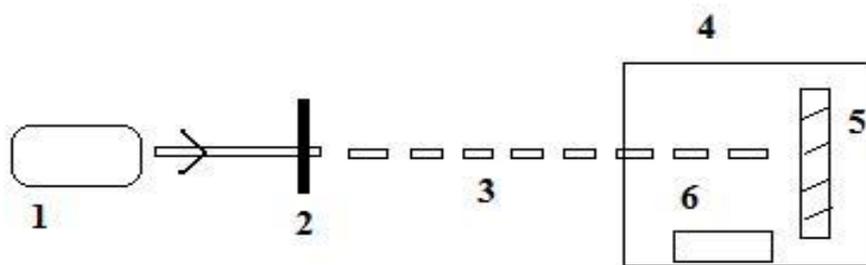
3.3. Photoacoustic cell

The Photoacoustic cell serves as a container for the sample under study and for the microphone or other device for the detection of the generated acoustic wave. An optimum design of the Photoacoustic cell represents a crucial point when background noise ultimately limits the detection sensitivity. In particular, for trace gas application many cell configurations have been presented including acoustically resonant and non-resonant cells, single and multipass cells, as well as cell placed intracavity. Nonresonant cells of small volume are mostly employed for solids samples with modulated excitation or for liquids and gaseous samples with pulsed laser excitation. As a unique example, a small volume cell equipped with a 'tubular' acoustic sensor consisting of up to 80 signal miniature microphone has been developed. These microphones are arranged in eight linear rows with ten microphones in each row. The row are mounted in a cylindrical geometry parallel to the exciting laser beam axis and located on a circumference around the axis. This configuration is thus ideally adapted to the geometry of the generated acoustic waves [20, 21].

3.4. Detection Sensors

As mentioned earlier the acoustic disturbances generated in the sample are detected by some kind of pressure sensor. In contact with liquid or solid samples these are piezoelectric devices such as lead zirconate titanate (PZT), $LiNbO_3$ or quartz crystals with a typical responsivity R in the range of up to V bar^{-1} or thin polyvinylidene-difluoride (PVF_2 or PVDF)-foils with lower responsivity. These sensors offer fast response times and are thus ideally adapted for pulsed photoacoustics [8].

For studies in the gas phase, commercial microphones are employed. These include miniature electrets microphones such as Knowles or Sennheiser models with typical responsivities (R_{mic}) of 10-20 mV Pa^{-1} as well as condenser microphones, e.g. Bruel & Kjaer models with typical R_{mic} of 100 mV Pa^{-1}. Usually R_{mic} depends only weakly on frequency [6, 7].

Fig. 2. Typical experimental arrangements used for Photoacoustic spectroscopy. (1) light source (2) the chopper (3) chopped light (4) photoacoustic cell (5) sample (6) Microphone (detection sensors)
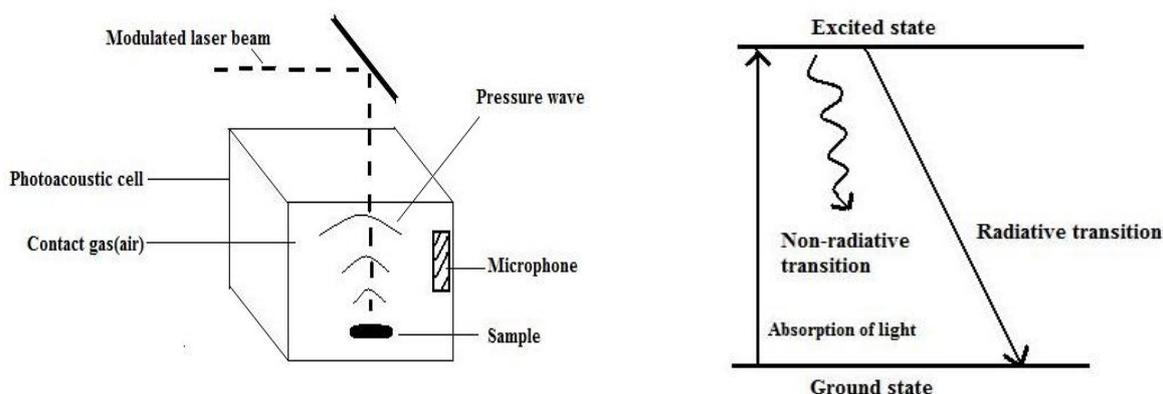
Fig. 3. Photoacoustic effect from a sample.

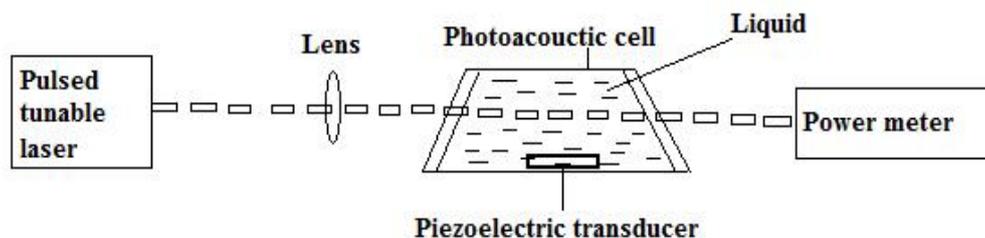## 4. Experimental Arrangement [6, 9-17]

As the photoacoustic and related photothermal phenomena comprise a large diversity of facets, there exist a various detection technique which rely on the acoustic or thermal disturbances caused by the absorbed radiation, the selection of the most appropriate scheme for a given application depends on the sample, the sensitivity to be achieved, ease of operation, ruggedness, and any requirement and any require for non-contact detection, e.g. in aggressive media or at a high temperature and/or pressure.

Experimental schemes for photoacoustic studies on solid sample includes the measurement of the generated pressure wave either directly in the sample with a piezoelectric sensor for the pulsed regime, or indirectly in the gas which is in contact with the sample by a microphone.
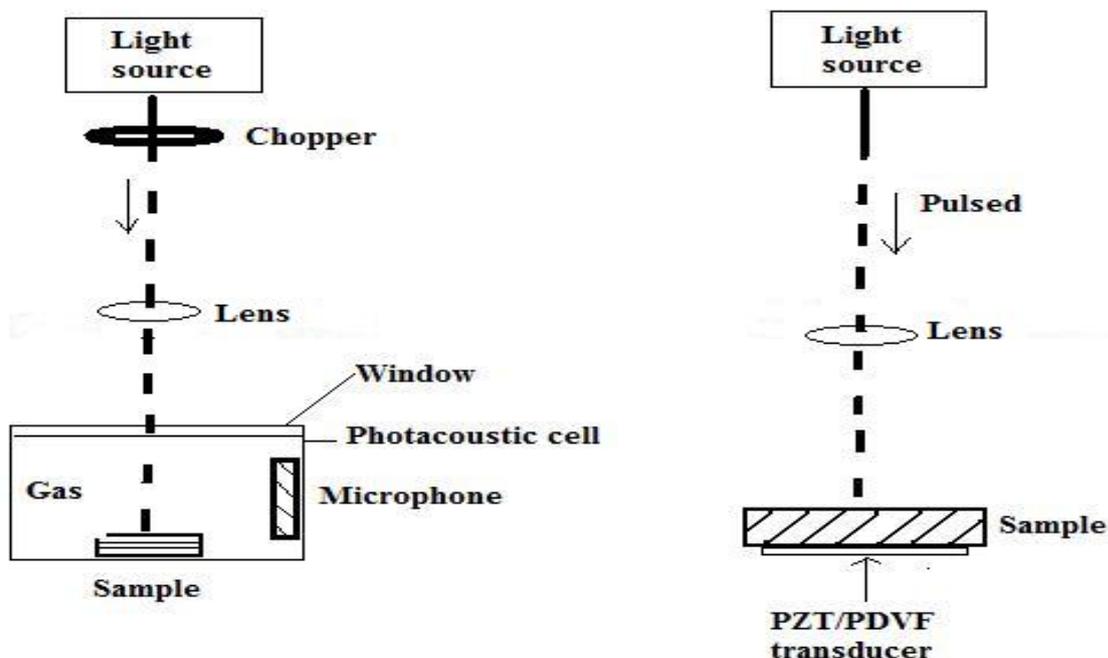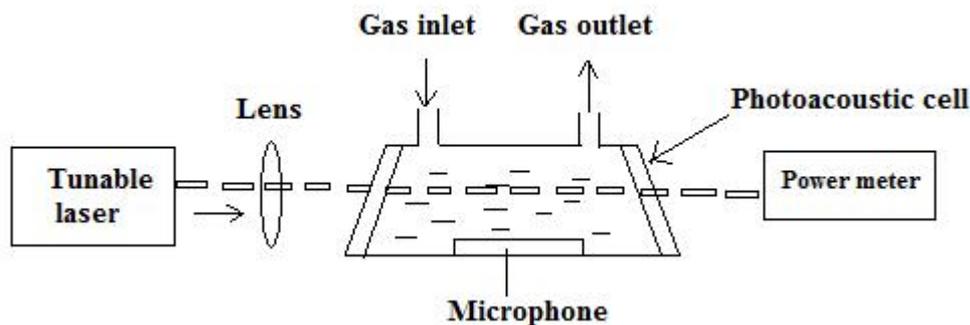

Fig. 4. Typical experimental arrangements used for Photoacoustic studies on solids.

The typical experimental arrangement for the absorption spectroscopy in weakly absorbing liquids is shown schematically in Figure 5. The beam of a pulsed tunable laser is directed through the PA cell that contains the sample under study. The generated acoustic waves are detected by a piezoelectric transducer with fast response time. Usually, only the first peak of the ringing acoustic signal is taken and further processed. Pulse-to-pulse variations of the laser power are accounted for by normalizing the piezoelectric signal with the laser power measured with the power meter after the cell.

Fig. 5. Typical experimental arrangements used for photoacoustic detection in liquids (PZT detection of acoustic wave generated by pulsed radiation in weakly absorbing liquids)

The typical setup for gas phase measurement is shown in Fig.6. A tunable laser with narrow line width or a conventional (broadband) radiation source followed by optical filters is used. In general, amplitude-modulated (or sometimes pulsed) radiation is directed through the PA cell. The acoustic sensor is usually a commercial electret microphone or a condenser microphone. These devices are easy to use and sensitive enough for trace gas studies with very low absorption. Often, the detection threshold is neither determined by the microphone responsivity (R_{mic}) itself nor by the electrical noise but rather by other sources (absorption by desorbing molecules from the cell walls, window heating, ambient noise, etc.). However, if this later background is known from reference measurements, the ultimate detection sensitivity is determined solely by fluctuations of the radiation intensity, and by microphone and amplifier noise. The frequency dependence of R_{mic} is usually rather small and the temperature dependence may have to be taken into account in special cases only. If modulated radiation is employed the microphone signal is fed to a lockin amplifier locked to the modulation frequency. If pulsed radiation is employed, the microphone bandwidth is often not sufficient to resolve the temporal shape of the generated acoustic pulses. However, common microphones can still be used even for nanosecond laser pulses because the length of a single acoustic pulse is essentially determined by the transit time of the acoustic wave across the beam radius.

Fig. 6. Typical experimental photoacoustic arrangements for gas monitoring with tunable laser sources.

5. Applications

5.1. Depth Profiling of Mammalian Cells for Localization of Ligands [23]

Phase-resolved monitoring of photoacoustic signals can provide information about the depth profile of a sample. The principle of photoacoustic spectroscopy has been used to determine the depth profiles of ligands and antitumor agents in mammalian cells. Measurements of the in-phase and quadrature components of the photoacoustic spectra (which yield information from the surface and the interior, respectively) of a tumor cell line, AK-5, treated with the antitumor agent coralyne chloride have been made. They clearly show that the drug accumulates in the cell interior and is not seen on the cell surface, providing in situ evidence for the localization of this drug. Histochemical dyes which stain cells uniformly give identical in-phase and quadrature spectra; spectra of cells incubated with nuclear stains demonstrate a differential staining of the nucleus and the cytoplasm. These results demonstrate the usefulness of phase-resolved photoacoustic spectroscopy in monitoring differential interactions of drugs and other ligands with cells.

5.2. The Biophysics approach: Photoacoustic spectroscopy to study the DNA-ethidium bromide interaction [24]

The binding processes of ethidium bromide interacting with calf thymus DNA using photoacoustic spectroscopy have been examined. These binding processes are generally investigated by a combination of absorption or fluorescence spectroscopies with hydrodynamic techniques. The employment of photoacoustic spectroscopy for the DNA-ethidium bromide system identified two binding manners for the dye. The presenceof two isosbestic points (522 and 498 nm) during DNA titration was evidence of these binding modes. Analysis of the photoacoustic amplitude signal data was performed using the McGhee-von Hippel excluded site model. The binding constant obtained was $3.4 \cdot 108$ M(bp)–1, and the numberof base pairs excluded to anotherdye molecule by each bound dye molecule (n) was 2. A DNA drug dissociation process was applied using sodium dodecyl sulfate to elucidate the existence of a second and weakerbin ding mode. The dissociation constant determined was 0.43 mM, whose inverse value was less than the previously obtained binding constant, demonstrating the existence of the weaker binding mode. The calculated binding constant was adjusted by considering the dissociation constant and its new value was $1.2 \cdot 109$ M(bp)–1 and the numberof excluded sites was 2.6. Using the photoacoustic technique it is also possible to obtain results regarding the dependence of the quantum yield of the dye on its binding mode. While intercalated between two adjacent base pairs the quantum yield found was 0.87 and when associated with an external site it was 0.04. These results reinforce

thepresence of these two binding processes and show that photoacoustic spectroscopy is more extensive than commonly applied spectroscopies.

5.3. Photoacoustic spectroscopy to study the presence of aromatic amino acid in proteins [25]

The examination of aromatic amino acid in six proteins with well known structures usingabsorption spectra of near ultraviolet PAS over the wavelength range 240–320 nm have been performed. The fundamental understanding of absorption of light and a subsequent release of heat to generate a transient pressure wave was used to test the concept of monitoring aromatic amino acids with this method. Second derivative spectroscopy in the ultravioletregion of proteins was also used to study the regions surrounding the aromatics and the percentage area in each band was related in order to determine the contribution in function of the respective molar extinction coefficients for each residue. Further investigation was conducted into the interaction between sodium dodecyl sulphate (SDS) andbothropstoxin-I (BthTx-I), with the purpose of identifying the aromatics that participate in the interaction. The clear changes in the second derivative and curve-fitting procedures suggest that initial SDS binding to the tryptophan located in the dimer interface and above 10 SDS an increased intensity between 260 and 320 nm, demonstrating that the more widespread tyrosine and phenylalanine residues contribute to the SDS/BthTx-I interactions. These results demonstrate the potential of near UV-PAS for the investigation of membrane proteins/detergent complexes in which light scattering is significant.

5.4. Photoacoustic spectroscopy as a key technique in the investigation of nanosized magnetic particles for drug delivery systems [26]

The study includes how cubic ferrite nanoparticles, suspended as ionic or biocompatible magnetic fluids, can be used as a platform to built complex nanosized magnetic materials, more specifically magnetic drug delivery systems. The study shows the use of the photoacoustic spectroscopy as an important technique in the investigation of key aspects related to the properties of the hosted nanosized magnetic particle. Examples of the use of the photoacoustic spectroscopy in accessing information regarding the particle size effect and the surface molecular coating of magnetic nanoparticle are included in the paper.

5.5. Analysis of Biological Material [27]

Conventional spectroscopy does not yield satisfactory spectra because of the string light scattering properties of the blood cells, protein and lipid molecules present. PAS permits spectroscopic studies of blood without the necessity of a preliminary separation of these large molecules.

Other biological application of PAS include identification of bacteria states, study of animal and human tissues including teeth, bone, skin, muscle etc., analysis of drug in tissues, investigating of the photo-oxidative decay in human eye lenses etc.

5.6. Evaluation of elasticity and integrity of pharmaceutical tablets [28]

A nondestructive method based on pulse photoacoustics was applied for evaluation of elasticity and integrity of pharmaceutical tablets. Variations in porosity, density and sodium chloride content of microcrystalline cellulose tablets were found to be related to parameters extracted from the through-transmitted ultrasonic wave forms. By using the amplitudes and ultrasonic velocities of these wave forms, it was possible to obtain values of a transverse to longitudinal amplitude ratio, and also elastic parameters, such as Young's and shear moduli,

for the tablets. The method is a promising tool for evaluating the elastic properties of tableting materials and the structural variations in tablets.

5.7. Quantitative analysis of drug content in semisolid formulation using step scan FT-IR Photoacoustic spectroscopy. [29]

Step-scan FT-IR photoacoustic spectroscopy in conjunction with a phase modulation technique (modulation frequency of 25 Hz) and digital signal processing was applied in order to quantify the content of brivudin and dithranol in vaseline/drug ointment. The PA spectra of the mixtures exhibit an excellent signal to noise ratio and bands belonging to the drugs are clearly observable down to an 0.5 wt% concentration of the drug. The integrated intensity of the drug bands with reference to a vaseline band was used as a measure of drug content. For comparison, the concentration of drug was determined by capillary zone electrophoresis for brivudin and by HPLC for dithranol. It appears that Beer's law is fulfilled for the PAS data. No sample preparation was required for the PAS experiments.

5.8. Monitoring electron transfer by photoacoustic spectroscopy [30]

The electron transfer process between octaethylporphin and quinone molecules dispersed in a polymeric matrix was studied by the photoacoustic technique. It was observed that there was an enhancement of the octaethylporphin photoacoustic signal with an increase of the quinone concentration in the films. This increase appeared to be complementary to octaethylporphin fluorescence quenching and was associated with the electron transfer process. The data were analyzed according to the theory developed by Kaneko for fluorescence data.

5.9. Gas Phase Analysis [31]

In recent years, the development of new PA setups for on-line gas monitoring has been achieved through new developments in diode lasers. Atmospheric pollutants that can be detected by PA measurement techniques includes sulfur oxides (such as SO_2), nitrogen oxides (NO_2), carbon oxides (CO and CO_2), hydrogen sulfide, ammonia, methane, and aerosol particles (such as soot).

5.10. Analysis of Condensed Matter [32]

The main application of UV/Vis.–PAS is the characterization of semi conducting materials. As the PA signal depends on heat diffusion, the thermal diffusivity can also be determined, which is strongly sensitive to the structural quality of the semi conducting material. Furthermore, packaging materials have been characterized by PA measurements in the UV/Vis range. Using depth-resolved PAS, it was possible to estimate the thicknesses and moisture contents of varnish layers on base paper.

5.11. Analysis of highly concentrated textile dyes using photoacoustic spectroscopy [21]

The concentrations of textiles dyes are in the range of more than 5 g L^{-1}, resulting in absorption coefficients of 10^3 cm^{-1}. The combination of extremely high absorption and scattering particles in the dye solution makes a classical transmission spectroscopic analysis impossible. PA spectroscopy is a viable approach to overcome the problems.

5.12. The malaria parasite monitored by photoacoustic spectroscopy [33]

Noninvasive photoacoustic spectroscopy was used to study the malarial parasites Plasmodium chabaudi and Plasmodium berghei, their pigment and ferriprotoporphyrin IX,

which is a byproduct of the hemoglobin that the parasite ingest. The result indicate that the pigment consits of ferriprotoporphyrin self aggregates and a noncovalent complex of ferriprotoporphyrin and protein. Spectra of chloroquine-treated parasites reveal in situ interaction between the drug and ferriprotoporphyrin. Chloroquine-resistant parasites, readily distinguishable by this method, appear to degrade hemoglobin only partially.

6. Conclusion

Photoacoustic spectroscopy (PAS) is based on optical absorption and subsequent detection of pressure fluctuations. The main advantages of this technique over conventional absorption spectroscopy are:

- Wide dynamic range (several orders of magnitude)
- Highly sensitive trace gas analysis with short pathlengths
- Measurement of high absorption coefficients, even in opaque samples, without sample dilution
- Determination of absorption spectra of solid samples, even in the form of powder, chips, or large objects
- Less influence from light scattering compared to conventional measurement techniques
- Depth profiling of layered samples

These features, allowing on-line measurements without sample pretreatment, are advantageous in process analysis. In the characterization of industrial products, additional benefits include the potential to determine absorption spectra of opaque solids and depth profiles of layered materials. For the analysis of gaseous, liquid, and solid samples, different combinations of excitation, signal generation and detection schemes have been developed. The most common PA techniques described in this article are:

1. Modulated excitation and direct generation of sound waves in gaseous samples
2. Modulated excitation of liquid and solid samples with subsequent indirect generation of sound waves in the adjacent gas phase
3. Pulsed excitation and direct generation of pressure pulses in liquid and solid samples.

Methods (1) and (2), based on modulated excitation and detection of sound waves by microphones, are derived directly from A.G. Bell's photoacoustic experiments in 1880. The first analytical applications of PAS in the 1960s also used this principle. Today, commercial gas cells and UV/Vis and IR spectrometers are available that are based on these old PAS methods. The main application of gas phase PAS to process analytical technology is in the monitoring of workplaces and industrial and diesel exhaust in order to optimize waste gas treatment, combustion and production processes. Indirect PA generation in solid and liquid samples is employed to characterize semiconductors, synthetic polymers, paper coatings, and fossil fuels. In recent years, PAS method (3), based on pulsed excitation and detection of pressure pulses, has been achieved through the development of new laser sources (such as tunable OPO systems) and detection methods (such as optical and interferometric detection). This technique is employed to investigate liquid samples, mainly due to its ability to determine absorption coefficients over a wide dynamic range, even in strongly absorbing and scattering samples. Sample pre-treatment is not necessary in most cases. The need for calibration can also be overcome by extracting absorption coefficients directly from the signal shape. Applications related to technical processes include the detection of hydrocarbon

contamination in water and the on-line monitoring of textile dyeing processes. Pulsed excitation in solid samples is used for both absorption measurements and depth profiling. Examples of the application of this include biofilm monitoring, measurement of painting thicknesses, and characterization of thin films and papers. As we have seen, PAS has been applied in many fields of process analysis and the characterization of industrial materials. PAS techniques based on modulated excitation have been used in analytical chemistry since the 1960s, and measurement systems are already available commercially. Recent developments in pulsed PAS – especially in piezoelectric and optical detection – open up new avenues of application in two-dimensional and three-dimensional tomography and contactless online measurement.

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