PHOTOACOUSTIC SPECTROSCOPY AS A METHOD IN BIOLOGICAL INVESTIGATIONS

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The present paper describes a new method of photoacoustic spectroscopy for the investigation of the properties of materials. This method is applied in studies of both inorganic and organic materials, including biological structures.

The paper also gives the physical basis of the method, the construction principle of the measuring apparatus and examples of application of photoacoustic spectroscopy in biology and medicine.

Photoacoustic spectroscopy is based on the phenomenon observed for the first time by Graham BELL in 1880 [1]. He observed that when irradiating the solid body with light pulses, the acoustic signals, corresponding to the sequence of light pulses, occurred. A similar phenomenon consisting in generation of acoustic waves in a gas contained in a chamber and irradiated by a chopped light beam was reported in 1881 by John TYNDALL [2] and Wilhelm ROENTGEN [3].

Theoretical works concerning this effect were developed in recent years, mainly due to ROSENCWAIG and GERSHO [4, 5, 6]. Since that time (the seventies) we have noted a rapid development of methods of photoacoustic spectroscopy (PAS) applied to investigate the absorption processes in solid state, in liquids and in gases.

The PAS methods allow one to obtain the absorption spectra (similar to optical ones) for substances in any phase, and in any form, like powders, gels, coloids etc.

A particular advantage of PAS methods is the possibility of registering absorption spectra of substances characterized by a very large coefficient of light attenuation, which was obviously impossible to achieve by optical spectroscopy methods. In Poland PAS methods were studied in order to apply them to investigate the physico-chemical properties of various materials [7, 8].

Photoacoustic spectroscopy consists in placing the studied sample in a closed chamber and irradiating it by chopped light. The molecule excited by a

strong laser beam or by a xenon lamp returns to its ground state via the radiative
or nonradiative disactivation processes. The nonradiative decay of excited states
results in the production of heat, which causes local change in the temperature
field T. This causes (e.g. during investigation of liquids) simultaneous local
changes in the density field ϱ, in the pressure field p, and in the molecular velo-
city field v. The state of the liquid before heating the particular region may be
regarded as the equilibrium state. while the local heating causes the pertur-
bation of this state.

Fluctuations of the density, pressure, molecular velocity and temperature
fields are the parameters which characterize this perturbation. These fluctua-
tions propagate from the region where the equilibrium is perturbed. When the
fluctuations of density and pressure satisfy the conditions of small amplitude

$$\frac{|\delta\varrho|}{\varrho_0} \ll 1; \qquad \frac{|\delta_p|}{p_0} \ll 1;$$

the equations of mass, momentum and energy conservation may be linearised.
Thus one obtains a set of linear differential equations describing the propa-
gation of the above mentioned perturbation as acoustical waves.

Assuming that there is no flow in the liquid being in the equilibrium state,
i.e. that $v_0 = 0$, we obtain equations of the following form:

— mass conservation equation

$$\frac{\partial(\delta\varrho)}{\partial t} + \varrho_0 \operatorname{div}(\delta v) = 0; \tag{1}$$

— momentum conservation equation

$$\varrho_0 \frac{\partial(\delta v)}{\partial t} + \operatorname{grad}(\delta p) - \eta \Delta(\delta v) - (\xi + \eta/3)\operatorname{grad}\operatorname{div}(\delta v) = 0; \tag{2}$$

— energy conservation equation

$$\frac{\partial}{\partial t}(\varrho_0 \delta E + E_0 \delta\varrho) + \operatorname{div}[\varrho_0 w_0 \delta v - \varkappa \operatorname{grad}(\delta T)] = 0; \tag{3}$$

where η — dynamical viscosity (internal friction), ξ — bulk viscosity, $\varkappa$ — coef-
ficient of heat conduction, δE — perturbation of internal energy with respect
to its equilibrium value, E_0 — equilibrium value of the specific internal energy,
w_0 — equilibrium value of the specific enthalpy.

Perturbation of the medium may be described by means of the Fourier inte-
gral of harmonic perturbations with respect to time. In this sense, one may
assume that the perturbation generates a beam of acoustic harmonic plane
waves. In the case when the effects of viscosity and heat conduction can be
neglected, and when one may assume that the propagation of perturbations

is an adiabatic process, then the wave equation for the perturbation δF of the physical quantity F is as follows:

$$\frac{\partial^2(\delta F)}{\partial t^2} = c^2 V^2(\delta F), \tag{4}$$

where c — the velocity of propagation of the perturbation, F — one of physicall quantities, ϱ, p, $\boldsymbol{v}$.

Considering the photoacoustic phenomenon one has to take into account the relations between three important factors: μ_a — the depth of light penetration into the investigated material; μ_s — depth of heat penetration, and l — thickness of the sample. Moreover, the power of the light source, the frequency of modulation of the light beam, the properties of the gas surrounding the sample in chamber, the state of the sample surface and the properties of the material which fastens the sample in the chamber, also influence the photoacoustic phenomenon.

In the PAS method one applies two basic types of measurement apparatus:
— spectrometers with a single light beam,
— spectrometers with a double light beam.

The prototype of the photoacoustic spectrometer was worked out at the Institute of Fundamental Technological Research. It is shown in Fig. 1. It is a single beam spectrometer with a xenon lamp being the source of exciting light.
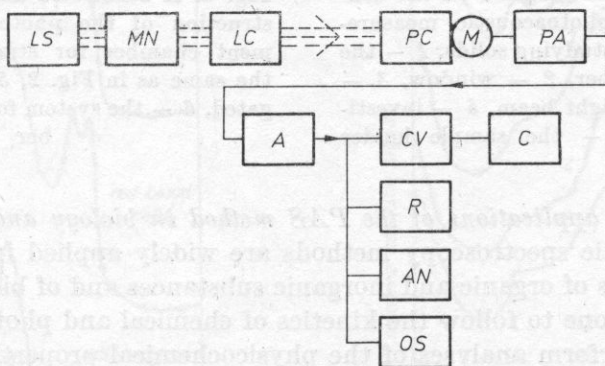

Fig. 1. A block diagram of the single beam photoacoustic spectrometer. LS — light source, MN — monochromator, LC — light beam chopper, PC — photoacoustic measurement chamber, M — microphone, PA — preamplifier, A — measuring amplifier, CV — analogue-to-digital converter, C — computer, R — registration apparatus, AN — spectrum analyzer, OS — oscilloscope

This spectrometer consists of three basic parts:
— light excitation system;
— measurement chamber with a microphone;
— electronic measuring and analyzing system.

The measurement chamber containing the sample of the material investigated is an essential part of the photoacoustic spectrometer. The acoustic receiving transducer is an integral part of this chamber. In the spectrometer prototype there is a possibility of applying exchangeable measurement chambers depending on the kind of investigated substance.

Figs. 2, 3 give examples of constructions of such chambers for investigating solids and liquids.
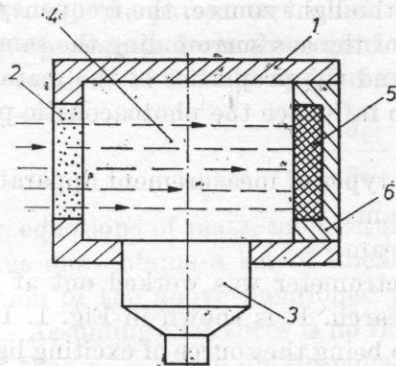

Fig. 2. A schematic diagram of the construction of the photoacoustic measurement chamber for studying solids. 1 — the body of the chamber, 2 — window, 3 — microphone, 4 — light beam, 5 — investigated sample, 6 — the sample holder
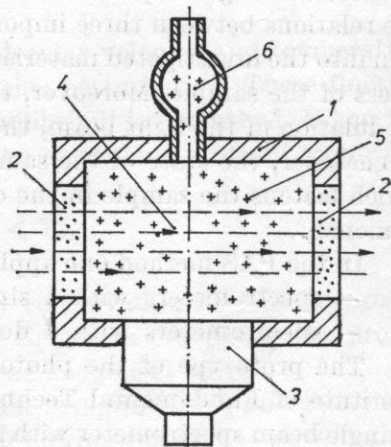
Fig. 3. A schematic diagram of the construction of the photoacoustic measurement chamber for studying liquids. 1-4 the same as in Fig. 2, 5 — liquid investigated, 6 — the system for filling the chamber

Examples of the applications of the PAS method in biology and medicine

Photoacoustic spectroscopy methods are widely applied in physicochemical investigations of organic and inorganic substances and of biological structures. PAS allows one to follow the kinetics of chemical and photochemical reactions, and to perform analyses of the physicochemical properties of materials. PAS enables one to detect vestigial guantities of substances like and groups, enzymes, metalic inclusions etc. Apart from technical applications, the PAS method is particularly advantageous in biological and medical studies. In this case it is possible to investigate dead and living tissues and organisms. The PAS method was applied to study the structure of samples, to estimate the growth of colonies of bacteria, to follow the development of tumours, etc. Examples of the photoacoustic spectra for sick and healthy human eye lenses are given in Fig. 4 [6]. Fig. 5 shows the PAS spectrum of whole blood [6].

The presence of protein and lipid material in whole blood, which causes so much difficulty in conventional spectroscopy from light scattering, creates
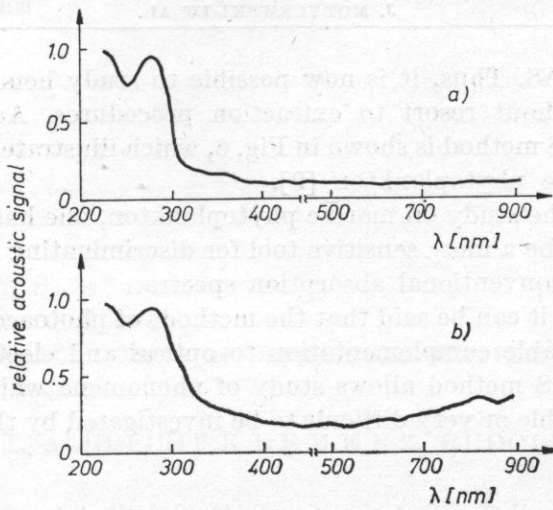
no problem in PAS. Thus, it is now possible to study acoustically directly, in whole blood, without resort to extraction procedures. Another example of the use of the PAS method is shown in Fig. 6, which illustrates the photoacoustic spectra of marine phytoplankton [9].

Concerning the above-mentioned examples, one has to admit that the PAS method can be a very sensitive tool for discriminating quite similar spectra than the conventional absorption spectra.

In summary, it can be said that the method of photoacoustic spectroscopy contains available complementation, too, to present and classical spectroscopic methods. The PAS method allows study of phenomena, which, in many cases, are either impossible or very difficult to be investigated by the existing conventional methods.

Fig. 4. The PAS spectrum of the lens of the human eye [6]. *a*) healthy lens, *b*) the lens with cataract
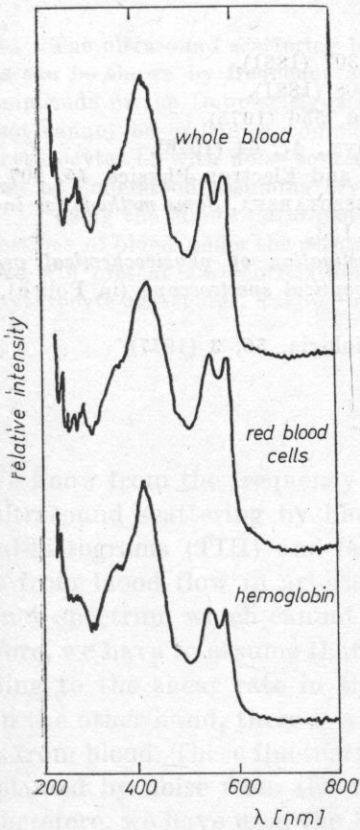

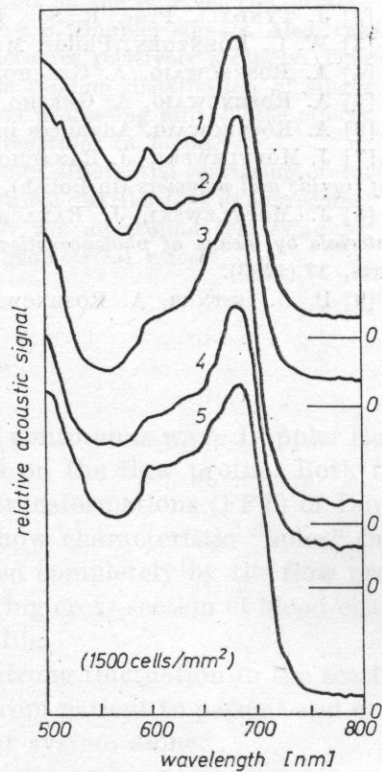Fig. 5. The PAS spectrum of smears of whole blood, red blood cells and hemoglobin [6]

Fig. 6. Photoacoustic spectra of representative marine phytoplankton [9]; *1* — thalasiosira, *2* — coccolithus, *3* — pyramimonas, *4* — dunaliella, *5* — platymonas

no problem in PAS. Thus, it is now possible to study hemoglobin directly in whole blood, without resort to extraction procedures. Another example of the use of the PAS method is shown in Fig. 6, which illustrates the photoacoustic spectra of marine phytoplankton [9].

Concerning the study on marine phytoplankton, one has to admit that the PAS method can be a more sensitive tool for discriminating quite similar speciments than the conventional absorption spectra.

In summary, it can be said that the methods of photoacoustic spectroscopy constitute a valuable complementation to optical and electrical spectroscopic methods. The PAS method allows study of phenomena which, in many cases, are either impossible or very difficult to be investigated by the existing conventional methods.

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