Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 127 (2014) 85–90

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Prediction of absorption coefficients by pulsed laser induced photoacoustic measurements

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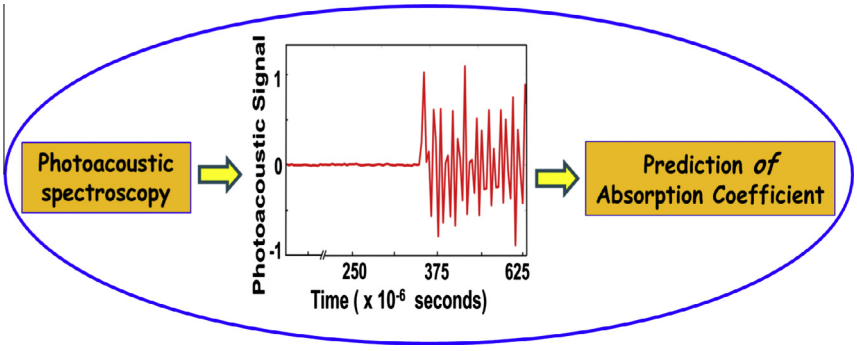
HIGHLIGHTS

- Pulsed laser induced photoacoustic spectroscopy setup was developed.
- A method for predicating absorption coefficients using the setup is proposed.
- Absorption coefficient determined for tryptophan concentrations & serum samples.
- Outcomes were cross-validated with spectrophotometric measurements.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form 4 December 2013
Accepted 9 February 2014
Available online 22 February 2014

Keywords:
Photoacoustic spectroscopy
PAS setup
Absorption coefficient
Tryptophan
Serum
Spectrophotometry

ABSTRACT

In the current study, a pulsed laser induced photoacoustic spectroscopy setup was designed and developed, aiming its application in clinical diagnostics. The setup was optimized with carbon black samples in water and with various tryptophan concentrations at 281 nm excitations. The sensitivity of the setup was estimated by determining minimum detectable concentration of tryptophan in water at the same excitation, and was found to be 0.035 mM. The photoacoustic experiments were also performed with various tryptophan concentrations at 281 nm excitation for predicting optical absorption coefficients in them and for comparing the outcomes with the spectrophotometrically-determined absorption coefficients for the same samples. Absorption coefficients for a few serum samples, obtained from some healthy female volunteers, were also determined through photoacoustic and spectrophotometric measurements at the same excitations, which showed good agreement between them, indicating its clinical implications.

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Introduction

Photoacoustic spectroscopy, has come a long way and established itself as an upcoming technology in the biomedical field because of its simplicity, highly sensitive and easy to handle qualities. The technique is widely being used in a variety of research areas, ranging from remote sensing for military purposes to detection of cancer, from trace element analysis in different materials to breath analysis. There is almost no field of science left where it has not been tested and found useful [1–9]. The principle behind this technique is that, when a modulated/pulsed light of specific wavelength is absorbed by a constituent of a sample, the constituent gets excited and upon de-excitation, the whole/part

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http://dx.doi.org/10.1016/j.saa.2014.02.021
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of the energy dissipates in the form of heat as non-radiative relaxations. These periodic heat fluctuations in the sample result in pressure wave or acoustic wave generation, which is then, detected using suitable pressure detectors (microphone or piezo-electric transducers). Over decades, the technique has witnessed a lot of new developments, leading to its application in tomography and imaging either in isolation or in combination with other biomedical tools such as ultrasound, sonography and microscopy [7,10–12]. The translational significance of this technique is majorly observed in the fields of biology, biotechnology and medicine for imaging of tissues and cells, detection of tumors in circulating cells, monitoring of body vasculature etc. [13–16]. There are also reports in the literature referred under this study that show development of new algorithms and statistical tools for analyzing photoacoustic data and extracting hidden information in them [17–20].

In photoacoustic spectroscopy, the resulting signal displays absorbed energy in terms of temporal amplitudes and phases, largely dependent on factors, such as, sample's physical properties (optical, acoustic, thermal), incident light fluence, photoacoustic cell dimensions and non-radiative relaxation processes [20–23]. The signal is the map of the amount of optical energy absorbed by the sample, and hence, it can be correlated to the product of optical absorption coefficient of the sample, and the incident light fluence [15,17,19,23]. In recent years, the usefulness of photoacoustic spectroscopy in determining optical absorption coefficients in various specimens, including biological samples, has been verified satisfactorily. The method followed for this purpose is either iterative fitting of the photon diffusion equation, employing point spread function (PSF) of the photoacoustic measurements [24] or the use of temporal amplitude and phase information of the photoacoustic signal along with the acoustic velocity within the sample [23–26]. There have been reports on work done on optical absorption coefficients for different seed genotypes (wheat, maize) [27,28] by photoacoustic measurements. Attempts have also been made to derive acoustic speed, thermal acoustic transformation coefficients in liquid samples, using photoacoustic spectroscopy [13–25,28–31].

The main idea behind the present study is to establish a facility based on pulsed laser-induced photoacoustic spectroscopy to evaluate biological variations in tissues, body fluids (serum and saliva) etc., subject to disease initiation. With this intension, we have established the setup through step-by-step development, achieving a very good sensitivity of minimum detectable concentration of 0.035 mM with tryptophan. The system has then been used successfully to determine the absorption coefficients for various tryptophan concentrations as well as for serum samples. The outcomes have later been compared with the corresponding spectrophotometric results.

Methodology

Experimental setup

The basic components of the experimental setup consisted of excitation source, photoacoustic (PA) cell, pre-amplifier and signal processing unit. The excitation source used was a combination of Nd-YAG laser (LM1278 LPY707G-10, LITRON Lasers, UK) and a dye laser system (PULSARE Pro, FINE ADJUSTMENT, Germany) with a frequency doubling option in it. The second harmonic (532 nm) output beam from the Nd-YAG laser was used to pump the dye laser containing Rhodamin 6G dye to provide lasing in the wavelength range of 545–580 nm. The dye laser output was then frequency doubled to get the required wavelength of 281 nm used in the present study. The next component, the PA cell which is the heart of the setup was designed and fabricated in-house as described previously [32]. The cell houses a sample holder and a detector (PZT in this case) coupled together. The sample holder is an arrangement for holding the cuvette containing sample with mechanical support provided by two rectangular stainless steel blocks. In the setup, in one of the blocks, an appropriate groove was carved for partial fixing of the cuvette. The PZT transducer was housed in the second block with Teflon isolation in such a way that nearly 1 mm of the PZT was always protruding beyond its surface. The PZT casting was made up of stainless steel to minimize any spurious signals generated from stray light absorption at the cell walls. The Teflon envelope containing PZT and the metal disc was then mounted onto the cylindrical metal base with threaded outer wall to fit in with the PZT housing. The metal casing enclosing the PZT cylinder minimizes the electrical pickups. Further, to reduce the acoustic reflection back into the transducer, the metal casing was soldered with lead material. The PZT (Model PIC 181, length 10 mm, diameter 5 mm, PI Ceramics, Germany) detector was then connected to the pre-amplifier using a BNC connector, which was further coupled to a Cathode Ray Oscilloscope (Tektronix, TDS 5034B) for signal processing and recording. The laser light from the Nd-YAG laser pumped dye laser was focussed onto the PA cell containing the sample in a quartz cuvette, held in between the stainless steel blocks of the cell using a 5 cm focussing lens. The block diagram of the experimental set up is shown in Fig. 1. The photoacoustic signal generated in the sample upon laser excitation was detected by the PZT detector which, upon further amplification using the pre-amplifier, was recorded on an oscilloscope. The oscilloscope recorded the photoacoustic data in time domain.

Determination of absorption coefficients by photoacoustic measurements

The experimental setup used to record photoacoustic spectra in the present study was first optimized using carbon black samples dissolved in Milli Q water and recording the corresponding photoacoustic signal at 281 nm excitation. Subsequently, detection limit of the system was evaluated using tryptophan concentrations. It is an established notion that absorption coefficient of a sample derived through photoacoustic measurements is proportional to its signal amplitudes [15–17,19]. When the logarithmic profile of the first temporal amplitude in time-resolved photoacoustic measurements for a sample under study is plotted as a function of its rise-time, the slope (S1) of the best fit line to the plot represents product of acoustic velocity (v) and the absorption coefficient (ϵ) for the sample [23–26,35] as shown in Eq. (1) below. Further, the acoustic velocity in a particular sample can be derived by varying the excitation-detector separation and by recording the

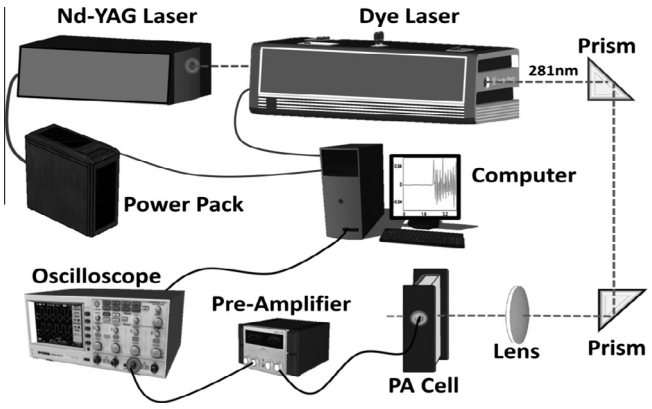

Fig. 1. Experimental block diagram of the photoacoustic spectroscopy setup.

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