Detection of thyroid stimulating hormone in human blood serum using photoacoustic spectroscopy

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ABSTRACT

The feasibility of measurement of Thyroid Stimulating Hormone in human blood serum, by photoacoustic spectroscopy is examined in the present study. In photoacoustic spectroscopy, light induced heat can be detected, which is produced during non-radiative de-excitation following absorption of light. In this study, the spectra of blood serum samples in the wavelength region of 350-750 nm were recorded. These serum samples were also analyzed by routine laboratory tests for Thyroid Stimulating Hormone. It was observed that the samples having high Thyroid Stimulating Hormone showed high absorption peak at 450 nm in photoacoustic spectra.

Key words: Thyroid stimulating hormone, photoacoustic spectroscopy, absorption peak, blood serum.

INTRODUCTION

In recent years, photoacoustic spectroscopy (PAS) has emerged as an effective research and analytical tool in many areas including, material science, chemistry, biotechnology and biochemistry (Nainwal and Kimothi, 2012). In PAS, the sample is periodically heated by the incidence of a chopped light beam and as a result, some energy levels in the sample are excited and these energy levels must subsequently de-excite, usually by means of non-radiative or heating mode of de excitation, and the pressure wave thus generated is detected by acoustic technique (Rosencaig, 1980; Zelya-Angel et al., 1994). Its advantage of detecting signal is purely due to the absorption of the optical energy by the sample alone. The generation of the photoacoustic signal can be considered in two parts: first the absorption of light to generate heat and second the diffusion of heat through the sample and adjacent medium to generate a thermal wave which can be detected.

Absorption of light and heat generation

The simplest case is absorption by an homogeneous sample which is uniformly illuminated by a harmonically modulated beam as described by McDonald et al. (1986). Then the beam intensity at any depth will be given by:

\[ I(x) = \frac{I_0}{2}\exp(\beta x)(1 + \cos \omega t) \]  \hspace{1cm} (1)

where \( I_0 \) is the intensity at the sample surface, \( \beta \) is the optical absorption coefficient; and \( \omega = 2\pi f \) where \( f \) is the modulation frequency. The heat produced (\( H \)) per unit volume will be:

\[ H = \eta \beta \frac{I_0}{2}\exp(\beta x)\exp(i\omega t) \]  \hspace{1cm} (2)

where \( \eta \) is the fraction of absorbed power converted to heat. However, the assumption of uniform irradiation is not always held when the pump beam size is smaller than the sample. Normally, the beam is Gaussian in profile and \( I_0 \) can be replaced with:

\[ I_{o}(r) = I_0 \exp(-2r^2/R^2) \]  \hspace{1cm} (3)

where \( r \) is the radial distance from the axis of a Gaussian beam of radius \( R \).
Diffusion of heat

The diffusion of generated heat is described by the equations of Carlslaw and Jaeger (1959):

\[-κV^2τ + ρC(δτ/δt) = H\]  \hspace{1cm} (4)

where, $κ$ is the thermal conductivity, $ρ$ is the density and $C$ is the specific heat. If the periodic time dependency of Equation 2 is introduced, then Equation 4 becomes:

\[V^2 r + iωτ/D_\text{th} = H/κδ\]  \hspace{1cm} (5)

where $D_\text{th}$ is the thermal diffusivity. Omitting the term $H$ and assuming uniform illumination, then a solution to this equation can be written as:

\[τ = A \exp (±qξ + iωt)\]  \hspace{1cm} (6)

here:

\[q = [kω/D_\text{th}]^{1/2}\]  \hspace{1cm} (7)

Thus, a thermal wave can be visualised whose amplitude decays by a factor of $1/e$ within a thermal diffusion length given by:

\[μ = [2D_\text{th}/ω]^{1/2}\]

Thyroid Stimulating Hormone (T.S.H.) promotes the growth of the thyroid gland in the neck and stimulates it to produce more thyroid hormones. Thyroid hormones affect three fundamental physiologic processes: cellular differentiation, growth, and metabolism (Duntas et al., 2003). Measurement of the levels of T.S.H. is considered a primary way to assess hypothyroidism and hyperthyroidism. T.S.H. is produced by the pituitary gland that stimulates the thyroid gland (Evered, 1974). When there is an excessive amount of thyroid hormones, the pituitary gland stops producing T.S.H., reducing thyroid hormone production. This mechanism maintains a relatively constant level of thyroid hormones circulating in the blood. Presently, ELISA (enzyme-linked immunosorbent assay) and RIA (radio immunoassay) tests are been used for T.S.H. detection. The detection of T.S.H. from these test requires skilled laboratory technicians and specialized laboratory equipment. In the present study, the potential of photoacoustic technique for detection of T.S.H. is explored.

EXPERIMENTAL

The Block diagram of the experimental set up used for the photoacoustic detection of T.S.H. is shown in Figure 1. The radiation from a 1000W Halogen Light source was made to pass through a chopper (Stanford research system, model SR-540) operating at 22 Hz. A particular wavelength was selected using Monochromator (central electronics limited, model HM 104). Photoacoustic (PA) cell equipped with a microphone (Knowles WP-23502) was used, where the test samples were placed. The electric signals from the microphone was fed to the Lock in amplifier (Stanford research system, model...
SR-530) synchronized with the chopper. Photoacoustic study was carried out at room temperature. The output from the Lock in amplifier for perfect absorber (carbon black) was taken and the PA spectrum (for carbon black) was recorded.

Forty serum sample were collected from Goyal Diagnostic centre, New Road, Dehradun, India. These serum samples given to diagnostic center by the suspected persons having hypothyroidism or hyperthyroidism. Samples were placed in a polystyrene tube and stored at -20°C until photoacoustic analysis.

For photoacoustic spectroscopy, 2 ml serum sample was placed inside a 3 mm inner diameter and 0.50 mm thick acrylic ring, whose bottom was blocked with 75 µm thick copper foil. The entire sample holder was placed on the top of the microphone and was attached by means of a thin layer of vacuum grease (Balderas-Lopez et al., 1995). The observed PA signal variation with empty copper foil is in perfect accordance with the model described by Rosencwaig and Gersho (1976).

Thus, the PA signal amplitude of each serum sample was recorded in the wavelength region of 350 - 750 nm, and it is divided, at each wavelength, by the corresponding value of the carbon black PA signal. The sample signal was thereby normalized against the lamp power spectrum at all wavelengths to provide a normalized spectrum. These serum samples were also analyzed by routine laboratory tests for T.S.H. The peak values of these samples were plotted and their trend was compared with the values reported by diagnostic centre.

RESULTS AND DISCUSSION

In this study, it was that the spectra showing high PA amplitude, had high T.S.H. value as reported by the diagnostic centre. For samples with the same T.S.H. value, they showed same PA amplitude. From Figures 2 and 3, it can be observed that the variation of peak value, closely matches with the data reported by pathology lab. Detailed study in this direction is in progress.

Conclusions

In conclusion, the trend observed in the plot of peak values of PA signal resembles that of pathology report. Correcting factors, which will remove the errors, need to be developed to improve the accuracy of reading. The present study has demonstrated that T.S.H. in the blood serum can be tracked using photoacoustic technique. Thus this technique appears as a promising tool for the detection of Thyroid Stimulating Hormone in the human blood serum. The potential of this non destructive technique could be successfully applied for detecting other hormones in the blood serum.
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