Drawing Energy Level Diagram of Blood Sample Using Nanosecond Laser Flash Photolysis

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Abstract. Nanosecond transient absorption experiments were performed using a laser flash photolysis (LFP) home built from Alneelain University Laser department (Sudan –Khartoum) with a Q-switched Nitrogen laser with a 337 nm output wavelength, 10 ns pulse width, 1 Hz repetition rate, and 40 mJ pulse energy. The optical detection is based on a pulsed Xenon arc lamp (75 W), spectra were recorded by Ocean Optics spectrometer and used origin 9 software to analysis data. Blood sample Laser flash photolysis experiments were performed with a 1 cm quartz flow cell mounted on a home-built sample holder that is placed at the cross-section of the laser incident beam and the probe flash lamp. The experiment was get the absorbance spectrum and drawing the energy level diagram of blood sample in three excited states, first excited state Start absorption band at 591 nm and end absorption band at 558 nm, second excited state Start absorption band at 562 nm and end absorption band at 517 nm and the last excited state Start absorption band at 463 nm and end absorption band at 390 nm.

Key words: Nanosecond, laser flash photolysis, optical detection, Nitrogen Laser, Home-Built.

Introduction

Flash photolysis is a commonly used fast reaction technique for photochemical reactions. For reactions with a moderate rate, flash lamps provide sufficient time response. An example of a typical flash lamp is the xenon lamp in a standard camera. For very fast reactions, however, the slow decay time of the light emission from a flash lamp covers the progress of the reaction. In general, the pulse width of the light source must be much shorter than the half-time of the chemical reaction (Beckett and Porter, 1963: 2038; Porter and Wilkinson, 1961: 1686; Changeux, 2012: 103-133). The pulse width of xenon flash lamps, such as those used in photography, is in the microsecond time scale. For faster reactions, specially designed lasers must be used that have pulse widths in the nanosecond range. Using ultra-fast pulsed lasers allows processes in the sub-femtosecond time scale to be studied. In our laser flash-photolysis system the lasers have pulse widths in the 10-nanosecond range, which allows a wide range of photochemical processes to be studied (DeWood et al., 1980: 897-902). One disadvantage of laser driven systems is that ultraviolet lasers have a fixed wavelength. Reactants in photochemical reactions can have a wide variety of absorption wavelengths, some of which may not be accessible to a given laser source. Therefore, several different types of lasers are often necessary to provide coverage of the UV range of common organic and inorganic reactants. To convert the IR light into the visible and then the UV region a special optical trick is used. Certain substances have non-linear optical properties in intense laser irradiation that combines the photons; doubling, then tripling,

and then quadrupling the photon frequency are possible. Potassium hydrogen phosphate is such a substance. Doubled output is at 532 nm, which is in the green region of the spectrum. Tripled output is at 355 nm and quadrupled at 266 nm. However, at each successive step the available power is greatly diminished. Output at 355 nm works well for many conjugated aromatic compounds. Excimer lasers use gas phase chemical reactions to provide highly excited diatomic molecules that emit light. The chemical reaction is initiated by an intense electrical discharge. The reaction used is normally between xenon and either fluorine or chlorine, producing either XeF or XeCl. The diatomic product is produced in a highly excited state with a lifetime in the nanosecond range. In dropping back down to the ground state, light is emitted in a short pulse. XeCl provides laser emission at 308 nm with a 0.3 nm spectral width and a pulse width of about 10 n sec. Many different techniques are available for monitoring the progress of photochemical reactions. Conductivity, IR, Raman, mass spectrometry, the aim of this work is to determine the absorbance spectrum of blood sample using an Ocean Optics Spectrophotometer (DeWood et al., 1983: 39-49; Lam et al., 2007: 1155-1159; Plohl, 2010: 42; Gopalakrishnan et al., 2005: 215-222).

The absorbance of a substance is given by the Beer-Lambert Law:

$$A = a\ b\ [A] \tag{1}$$

where a is the molar absorption coefficient, b the length of the optical path within the solution, and [A] is the concentrations (Zensen et al., 2016: 1600238). (Please note that [A], in brackets, is the concentration of species A, while A, without brackets, is the absorbance of the solution.] If other species that absorb at the same wavelength are in solution that are not involved in the reaction, then a constant background absorbance must be added to find the total absorbance of the solution:

$$A_{tot} = A + A_{background} = a\ b\ [A] + A_{background} \tag{2}$$

This equation can be solved to find the concentration of the species of interest:

$$[A] = \frac{A_{tot} - A_{background}}{ab} \tag{3}$$

If the molar extinction coefficient is not known the absorbance can be used directly in curve fitting. For a first order reaction:

$$\ln [A] = -\ k\ t + \ln [A]o \text{ or } \ln \frac{A_{tot} - A_{background}}{ab} = -\ k\ t + \ln [A]o \tag{4}$$

The curve fit can then be done with $\ln (A_{tot} - A_{background})$ as the y–variable:

$$\ln (A_{tot} - A_{background}) = -\ k\ t + \ln [A]_o + \ln (a\ b) \tag{5}$$

The constants a and b just become part of the intercept for the curve fit:

$$\ln (A\ tot - A\ background) = -\ k\ t + cst \quad \text{with } cst = \ln [A]o + \ln (a\ b) \tag{6}$$

For a second order reaction:

$$\frac{1}{[A]} - \frac{1}{[A]_o} = kt \quad or \quad \frac{1}{\frac{A_{tot} - A_{background}}{ab}} - \frac{1}{\frac{A_{tot(0)} - A_{background}}{ab}} = kt \tag{7}$$

Where A tot (0) is the initial absorbance of the solution. The curve fit can then be done with 1/(A tot – A background) as the y–variable:

$$\frac{1}{\frac{A_{tot} - A_{background}}{ab}} = \frac{k}{ab}t + \frac{1}{\frac{A_{tot(0)} - A_{background}}{ab}} \tag{8}$$

Unfortunately, the slope does not give the rate constant directly. But the rate constant is proportional to the slope. As long as this proportionality is kept in mind, the slope can be considered as an effective rate constant for comparison from solution to solution (Spilotros et al., 2012: 6434-6437).

Three separate experiments will be performed and the results combined to get the absorbance spectrum drawing the energy level diagram of blood sample using an Ocean Optics Spectrophotometer.

Materials and Method

Components necessary for building the flash photolysis setup are available in Alneelain University. These are:

- Laser sources like N2 laser (337.1 nm).
- A set of flash lamps is also available in our lab Xe (75 W power)
- A compact spectrometer of the type USB2000 from Ocean optic company with light resolution (1.34 nm, FWHM) is also available.
- Analysis of data will be performed using origin 9 software.

Building the flash photolysis setup like as showing in Fig. 1, then collected data used in this work, and analysis the data by origin 9 software

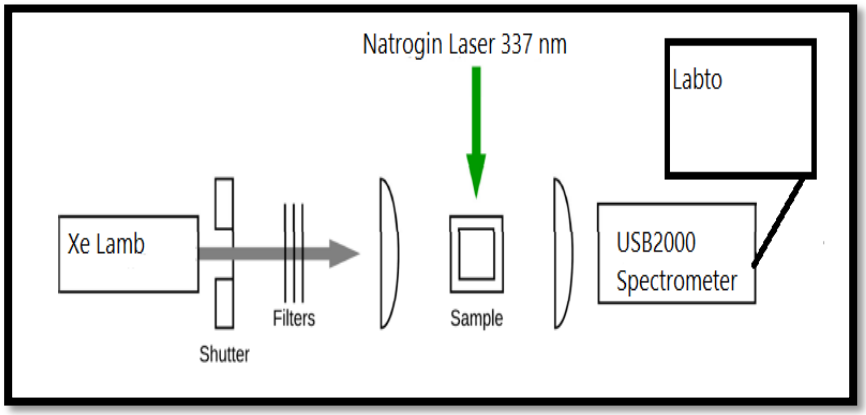

Fig. 1. Home Building flash photolysis setup

Determine the absorbance spectrum of blood sample. Print and save your spectrum. Use a quartz cuvette. Determine the absorbance spectrum using Ocean optic USB 2000 spectrometer. Using the automated data analysis software ruins all the fun of doing kinetics calculations. In this part of the calculations, you will construct a spreadsheet to repeat the automatic calculations. Transfer your raw data file into origin. Use all equation in part one to make absorbance spectrum. Then determine the energy level diagram of blood sample using the automated software.

Results and Discussion

Outline of this work are two figures Fig. 2 is relationship between absorbance and wave length, and Fig. 3 vibrational transitions to a given electronic state energy.

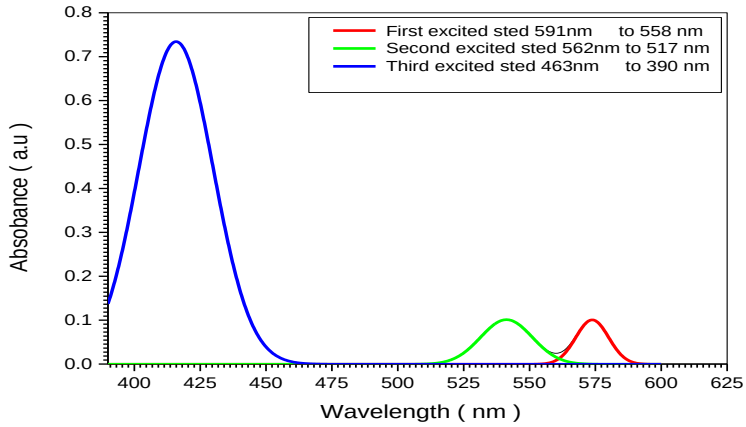

Fig. 2. Absorbance spectrum of blood sample by flash photolysis method

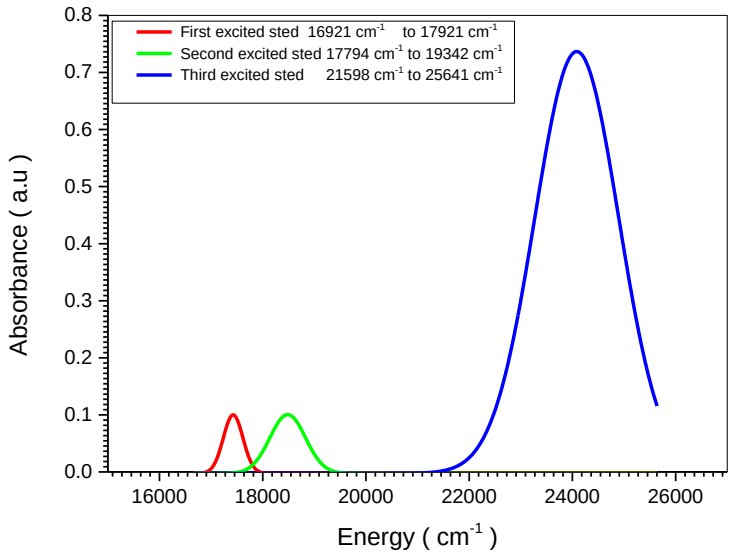

Fig. 3. Energy level diagram of blood sample calculations by flash photolysis method and software

Table's 1 start and end of each band are read from the deconvoluted spectrum. The values are approximate and are often read in nm from the original spectrum and converted to wavenumbers.

Table 1. Start and End of Absorption Band Data

Transition	Start of absorption band λ (nm)	cm^{-1}	End of absorption band λ (nm)	cm^{-1}
First excited state	591	16921	558	17921
Second excited state	562	17794	517	19342
Third excited state	463	21598	390	25641

In Fig. 2 is shown the relation between absorbance and wavelengths for blood sample, in the ranged (390 - 600) nm. Also in Fig. 1 show that three effective absorbance's area, first area at (591 – 558) nm ranged, second area at (562 – 517) nm and the last area at (463 – 390) nm. The resulting energy level diagram is shown in Fig. 3. The process for drawing the energy level diagram can be illustrated by picturing the spectrum tilted on its side. The different excited state bands are offset for clarity (they are all singlet states if the ground state is a singlet). Each electronic transition is really a set of transitions to different vibrational states of the same electronic state. The set of vibrational transitions to a given electronic state form a band of states given by the width of the electronic transition. The first step is to convert the wavelengths to energy units or units like cm^{-1} that are directly proportional to energy, Fig. 3. The process of drawing the energy level diagram can be illustrated simply by rotating the absorbance spectrum on its side and using the spectral transitions to delineate the energy levels into bands. It is common for the transitions to overlap. Table 1 provides the energies that are needed for this process. The wavelengths or wavenumbers at the start and end of each band are read by eye directly from the de convoluted spectra, plotted verses either wavelength or wavenumber. The resulting energy level diagram is shown in Fig. 3. Each electronic transition is really a set of transitions to different vibrational states of the same electronic state. The set of vibrational transitions to a given electronic state form a band of states given by the width of the electronic transition.

Conclusion

In the present paper, we have set vibrational transitions to a given electronic state form a band of blood sample in three energy band, first band at (16921-17921) cm^{-1}, second band at (17794-19342) cm^{-1} and the last band at (21598-25641) cm^{-1}.

References

Beckett, A., Porter, G. (1963). Primary photochemical processes in aromatic molecules. Part 10. Photochemistry of substituted benzophenones. Trans. Faraday Soc., 59, 2038. https://doi.org/10.1039/TF9635902051

Changeux, J.-P. (2012) Allostery and the Monod-Wyman-Changeux Model after 50 YearsAnnu. Rev. Biophys., 41, 103-133. https://doi.org/10.1146/annurev-biophys-050511-102222

DeWood, M.A., Spores, J., Hensley, G.R., Simpson, C.S., Eugster, G.S., Sutherland, K.I., Grunwald, R.P., Shields, J.P. (1983). Coronary arterio graphic findings in acute trans mural myocardial infarction. Circulation, 68(2 Pt 2), 39-49.

DeWood, M.A., Spores, J., Notske, R., Mouser, L.T., Burroughs, R., Golden, M.S., Lang, H.T. (1980). Prevalence of total coronary occlusion during the early hours of trans mural myocardial infarction. N Engl J Med., 303(16), 897-902. https://doi.org/10.1056/NEJM198010163031601

Gopalakrishnan, P., Kazmierczak, M.J., Banerjee, R.K. (2005) Thermal Interaction between laser & tissue during retinal photocoagulation. Proceedings of the SPIE, 5688, 215-222. https://doi.org/10.1117/12.606276

Lam, R.C., Shah, S., Faries, P.L., McKinsey, J.F., Kent, K.C., Morrissey, N.J. (2007). Incidence and clinical significance of distal embolization during percutaneous interventions involving the superficial femoral artery. J Vasc Surg, 46(6), 1155-1159. https://doi.org/10.1016/j.jvs.2007.07.058

Plohl, G. (2010). Mentor: dr. Rok Petkovšek - Pulsed Laser ablation. Ljubljana. Available at: http://mafija.fmf.uni-lj.si/seminar/files/2009_2010/Pulsed_Laser_Ablation.pdf

Porter, G., Wilkinson, F. (1961). Primary photochemical processes in aromatic molecules. Part 5.—Flash photolysis of benzophenone in solution. Trans. Faraday Soc., 57, 1686. https://doi.org/10.1039/TF9615701686

Spilotros, A., Levantino, M., Schirò, G., Cammarata, M., Wulff, M., Cupane, A. (2012). Probing in cellprotein structural changes with time-resolved X-ray scattering. Soft Matter, 8, 6434-6437. https://doi.org/10.1039/C2SM25676B

Zensen, C., Fernandez, I. E., Eickelberg, O., Feldmann, J., Lohmüller, T. (2016). Detecting Swelling States of Red Blood Cells by "Cell-Fluid Coupling Spectroscopy". Advanced science (Weinheim, Baden-Wurttemberg, Germany), 4(2), 1600238. https://doi.org/10.1002/advs.201600238