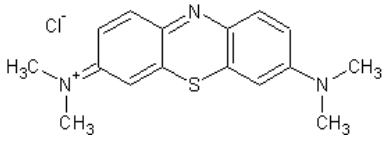
Spectral-Kinetic Properties and Pharmacological Treatment of Methylene Blue in Endoscopy and Photodynamic Therapy of Precancerous Lesions

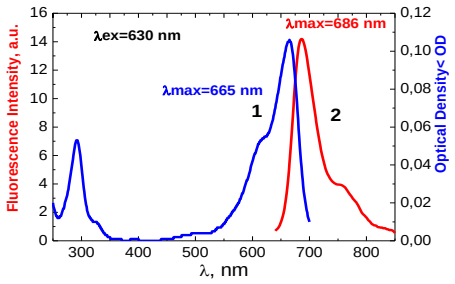
Prof. Eduard .I. Zenkevich[1],e-mail: zenkev@tut.by
T.A. Gin'ko[2], M.V. Parkhats[3], A.S. Stashevsky[3], A.P. Stupak[3],
Jin Young Jeong[4], Bong Hyun Chung[4]

[1]National Technical University of Belarus, Minsk, Belarus
[2]Minsk CIty Clinical Oncologic Dispensary, Minsk, Belarus
[3]B.I. Stepanov Institute of Physics, National Academy of Sciences, Minsk, Belarus
[4]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

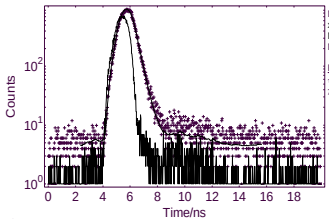
Methylene Blue (MB) is a molecule that has been playing important roles in microbiology and pharmacology for some time. It has been widely used to stain living organisms, to treat methemoglobinemia, and lately it has been considered as a drug for photodynamic therapy (PDT). In this talk, we present the fundamental photophysical, photochemical and photobiological characteristics of this molecule and evolve to show for the first time *in vivo* applications related to PDT of precancerous lesions (stomach upper gastrointestinal tract) based on endoscopic technique.



Methylene Blue (MB)



Absorption and Fluorescence Spectra



Fluorescence Decay

It is well-known that PDT is a promising modality for the management of various tumors and nonmalignant diseases based on the combination of a photosensitizer (PS) that is selectively localized in the target tissue and illumination of the lesion with visible light, resulting in photodamage and subsequent cell death. The main photophysical process leading to the singlet oxygen ($^1\Delta_g$) formation is the energy transfer from the triplet excited PS to the triplet oxygen:

$$^3MB^{+*} + O_2 \xrightarrow{k_2} MB+ + {}^1O_2 .$$

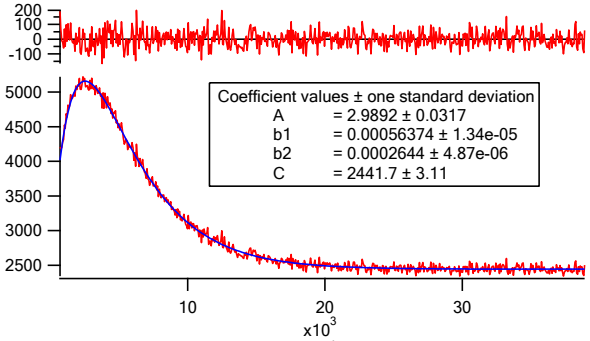
Measurements of spectra and decays (using TCSPC approach) of the singlet oxygen emission (λ_{max} = 1270 nm) as well as quantum efficiencies of $^1O_2$ generation (γ_Δ) have been performed on laboratory highly sensitive laser NIR spectrometer [1-3].: laser excitation by STA-01SH Nd:LSB laser (λ_{exc} =531 nm, energy of 4 μJ, FWHM = 0.7 ns, repetition rate of 1 kHz. STANDA Ltd.), monochromator MS2004i, (registration range of 950÷1400 nm, SOLAR TII Ltd.), computer photon counting board P7888-2 board (time resolution of 1ns/channel, FAST ComTec GmbH), PMT Hamamatsu H10330-45, (experimental response $\Delta t_{1/2}$ = 1 ns). The main procedure for the measurement of $^1O_2$ generation quantum efficiency γ_Δ^x is based on the comparison of emission intensity of singlet oxygen (λ_{max}=1.27 μ) photosensitized by a standard compound (intensity I_0) and by MB (intensity I_X) in the same solvent

$$\gamma_\Delta^x = \gamma_\Delta^0 \frac{I_x \times \beta_0}{I_0 \times \beta_x},$$

where γ_Δ^0 is the quantum efficiency of singlet oxygen generation by a standard, $\beta_0 = (1\text{-}10^{-D0})$ and $\beta_x = (1\text{-}10^{-DX})$ are fractions of absorbed exciting light by the standard and MB, respectively, at a given excitation wavelength. I_x and I_0 values were averaged and extrapolated to the maximal pulse intensity after more than 32 laser pulses for every measurement.

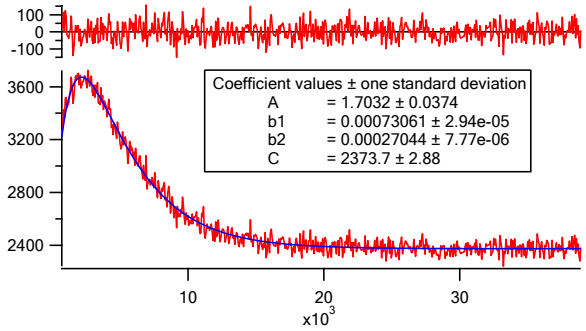
It was shown that in homogeneous solution where no dimers are present (ethanol or diluted aqueous solutions) MB produces triplets with high quantum yield (γ_{ISC}= 0.52), working as a $^1O_2$ photogenerated source γ_Δ~0.5) in comparison with a standard (water-soluble porphyrin $TMPyP^{+4}$ having the efficiency of singlet oxygen generation γ_Δ^0 = 0.77 [4]). In aqueous solution the efficiency of $^1O_2$ is dependent on the pH. MB triplets are excited state bases, so that its pK_a increases from a negative value in the ground state to around 7.5 in the triplet state. Therefore, the pH of the solution may certainly affect the efficiency of type I and type II photosensitization mechanisms. From PDT treatment point of view the MB main absorption band are in the therapeutic window like a lot of phosensitizers based on tetrapyrrolic compound.

Some experimental results on singlet oxygen generation *in vitro* are presented below:



Standard $MPyP^{4+}$ in H_2O

$\tau_\Delta \approx 3.78$ μs, $\tau_T \approx 1.77$ μs

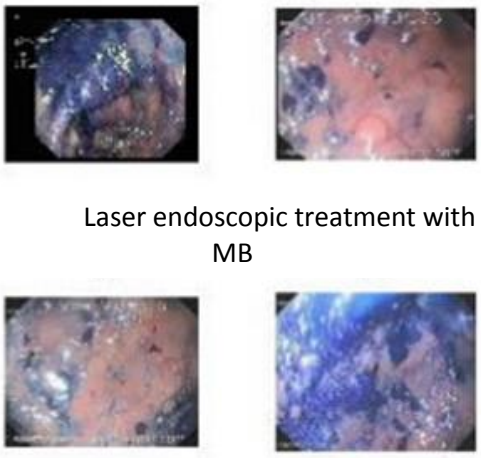


MB in H_2O

$<\tau_\Delta> \approx 3.64$ μs, $<\tau_T> \approx 1.39$ μs

PDT of precancerous lesions for patients has been carried out using endoscopic technique (GIF «OLYMPUS», type XQ-30, Q-40, video informative system V-70, two-channel endoscope with frontal optics). Efficiencies of singlet oxygen $^1\Delta_g$ generation (in water solutions) as well as diagnostics abilities and PDT efficiencies in precancerous lesions (stomach upper gastrointestinal tract) have been compared for MB (widely known histological dye) and for Photolon (developed by the Scientific Pharmaceutical Center of RUE Belmedpreparaty, Belarus). It has been shown that with respect to Photolon, MB has interesting characteristics (high water solubility, low toxicity, high efficiency of singlet oxygen emission γ_Δ=0.43 in water) conferring to this molecule a great potential for application in PDT.

We have found also that in addition to a pronounced diagnostic factor, MB is effectively concentrated in cancer cells and upon laser excitation at λ_{exc}=670 nm and 630 nm triggers the photosensitization mechanism of necrosis. It was estimated that the necessity of the chromoscopy and PDT of the precancerous lesions of stomach upper gastrointestinal tract with Photolon and MB is ≥ 26%.



Laser endoscopic treatment with MB

Acknowledgment: Belarussian State Research Program "Convergence 3.2.08" and Moscow Representative Office of National Research Foundation of Korea are greatly acknowledged for partial financial support.

References

1. V.A. Galievsky, A.S. Stasheuski, V.V. Kiselyov, A.I. Shabusov, M.V. Belkov, B.M. Dzhagarov. Instruments and Experimental Techniques, 2010, 53, 568.
2. E.I. Zenkevich, E.I. Sagun, V.N. Knyukshto, A.S. Stasheuski, V.A. Galievsky, A.P. Stupak, T. Blaudeck, C. von Borczyskowski. Journal of Physical Chemistry C. 2011, 115, 21535.
3. E.I. Zenkevich, S.V. Gaponenko, E.I. Sagun,and C. von Borczyskowski. Bioconjugates based on semiconductor quantum dots and porphyrin ligands: properties, exciton relaxation pathways and singlet oxygen generation efficiency for PDT applications. Reviews in Nanoscience and Nanotechnology, American Scientific Publishers, USA, 2013, v. 2, No 3, 184-207.