AN OPTICAL METHOD FOR MONITORING OF PHOTODYNAMIC INACTIVATION OF BACTERIA

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Abstract: *Photodynamic inactivation (PDI) is based on the interaction of light with a photosensitizer and molecular oxygen. In this way, reactive oxygen species (ROS) are generated, causing death of the bacteria. The efficacy of this antimicrobial therapy is evaluated usually, by using the standard microbiological methods for determination of the viability or colonies-forming units. An optical method for monitoring of photodynamic inactivation of bacteria especially for Staphylococcus aureus and Pseudomonas aeruginosa, is presented in this paper based on the determination of the refractive index of bacteria. By using Kramers-Kronig analysis of the reflectance spectra for different bacterial culture cells, it is possible to determine the optical parameters (refractive index and absorption coefficient) of the bacterial cultures. This method could be an important tool for management and evaluation of in vivo photodynamic inactivation of bacteria. It is a correlation between the number of bacterial colonies and refractive index of bacterial cultures, which means that based on a curve of calibration it can be, determined the number of colonies from measurements of diffuse reflectance*

Introduction: Microscopic pathogens are widely spread in nature, numerous infection sources do exist and the unfit and prolonged antibiotic treatments have lead to greater germ resistance to these substances. The permanent selection of new strands of antibiotic resistant bacteria is nowadays a major problem in both human and veterinary medicine. In this context modern researches were oriented to developing new methods of antimicrobial therapy, more efficient and faster, noninvasive and nontoxic, which do not lead to microbial resistance . One of these noninvasive methods is photodynamic inactivation of bacteria. Although only experimental stages are known up to now, there are remarkable results in killing by photodynamic inactivation of germs, which generate several types of infections [1-9]. In these studies, the efficiency of antimicrobial therapy is evaluated by using the standard microbiological methods for determination of the viability or colonies-forming units. These methods imply the prelevation of biopsic are difficult and time-consumer. A time-monitoring method of photodynamic action of light on *Staphylococcus aureus* and *Pseudomonas aeruginosa* and Toluidine Blue as photosensitizer are discussed in this paper. This optical method is based on the determination of optical parameters variations of bacteria during photodynamic inactivation.

Materials and method:

- *Bacterial cultures:*

  Staphylococcus aureus (2,5 x 10 9 CFU/ml)
  Pseudomonas aeruginosa (1,7 x 10 9 CFU/ml)

- *Photosensitizers* : Toluidine Blue (TBO), cTBO = 8.67x10-3M and pH = 7.4

- *Light sources:* Laser system SCL (INOE 2000, Bucharest, Romania): λ = 635 nm, P = 15 mW

- *Photodynamic inactivation:* From the initial cultures of the 2 bacterial species there have been distributed 5 ml in each of the 5 Petri dishes, with a diameter of 10 cm. They were put in contact with 5 ml of TBO and incubated in the dark for 15 minutes, and afterwards irradiated for 20 minutes at a distance of 1,5 cm.

- *Methods for monitoring of photodynamic inactivation of bacteria*
  Diffuse reflectance spectroscopy
  Kramers-Kronig analysis

$$k(\omega)=\frac{-2\sqrt{R(\omega)}\cos\varphi(\omega)}{1+R(\omega)-2\sqrt{R(\omega)}\cos\varphi(\omega)}$$

$$n(\omega)=\frac{1-R(\omega)}{1+R(\omega)-2\sqrt{R(\omega)}\cos\varphi(\omega)}$$

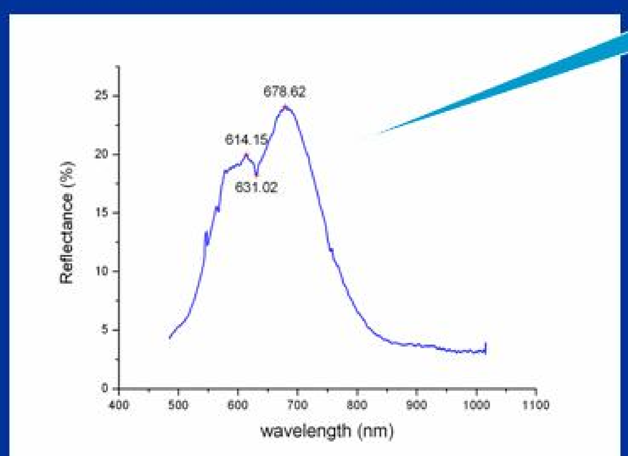
Results:

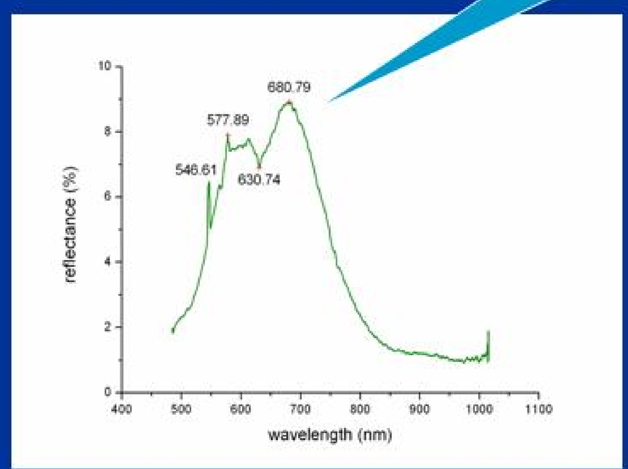
Diffuse reflectance spectroscopy:

before laser irradiation (without TBO added)

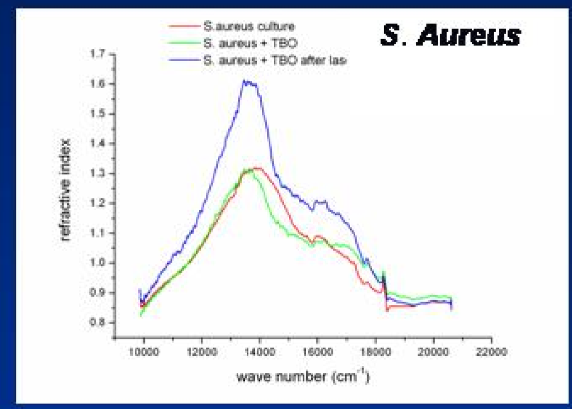
S. aureus culture has 2 reflectance maxima at: 614.15 nm and 681.07 nm

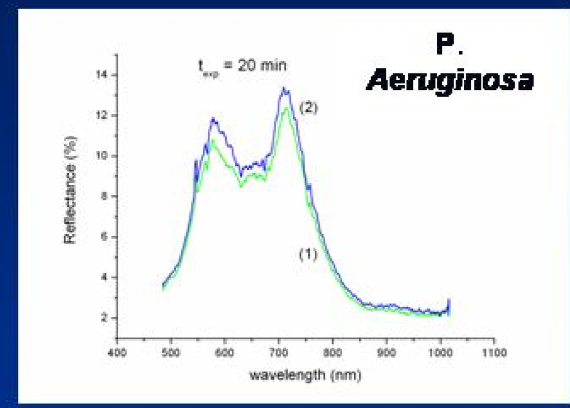
P. aeruginosa culture has 3 reflectance maxima at: 546.61 nm, 576.89 nm and 680.79 nm.





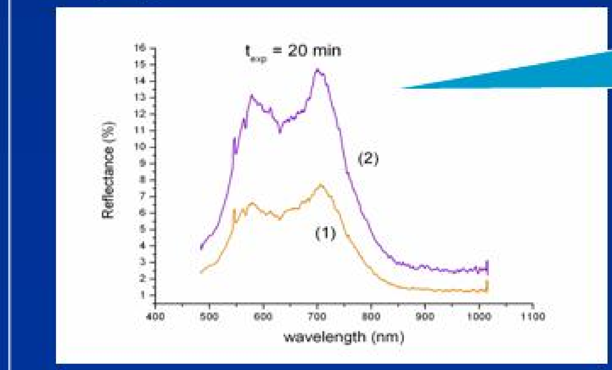
after laser irradiation



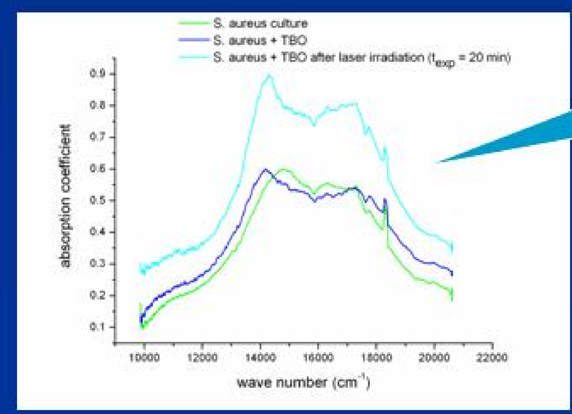


Kramers-Kronig analysis

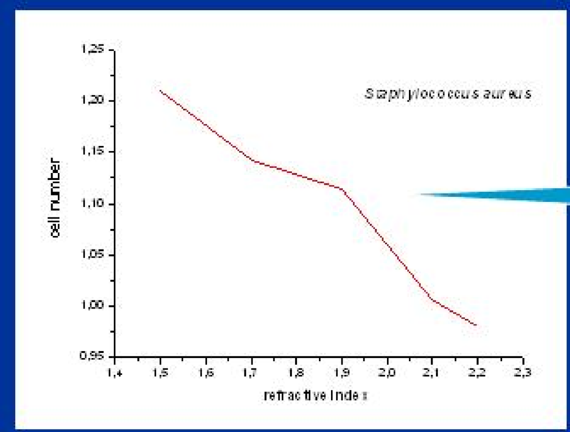
Staphylococcus aureus culture



Before TBO added n = 1.0729 (λ = 635 nm)
After TBO added n=1.059
After laser irradiation n↑and CFU↓



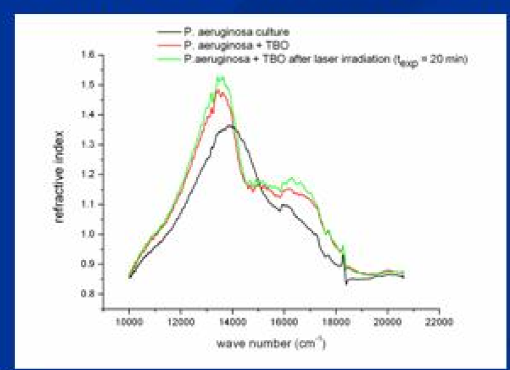
Before TBO added k = 0.5345 (λ= 635 nm)
After laser irradiation k↑ and CFU ↓

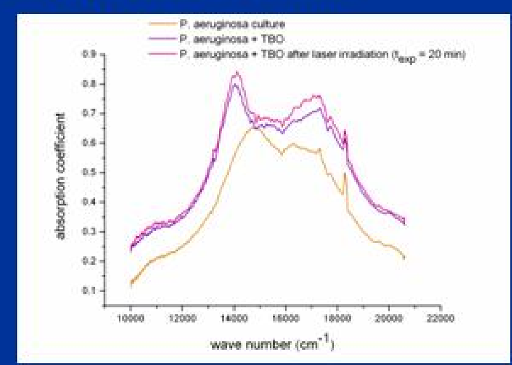


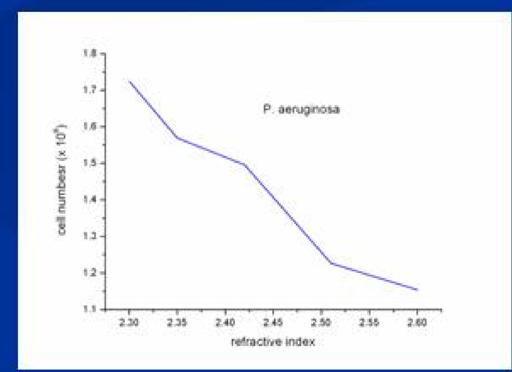
There is a correlation between the number of bacterial colonies and refractive index of bacterial cultures. Based on a curve of calibration it can be determined the number of colonies from measurements of diffuse reflection.

Pseudomonas aeruginosa cultures:

The optical parameters n(ω) and k(ω) of *Pseudomonas aeruginosa* cultures at 635 nm present the same behavior like *Staphylococcus aureus* cultures.







Conclusions: Application of photodynamic inactivation of bacteria in order to treat infections has some unknown fields: which are the bacteria with sensibility to light radiation, the direct effect on the microbial population, the type of photosensitizer which is selectively fixed in different bacterial spp., the way it should be administered, how to prepare photosensitizer, what is the therapeutic concentration, how much time must pass from the photosensitizer administration to the exposal to the light, which type of source for the radiation is better (continuous or pulse operation), the parameters of the light (wavelength, energy, pulse duration, frequency, time of exposure), how to monitor the biologic response and the treatment. Regarding this last problem, for bacteria photodynamic inactivation, up to now has been applied standard microbiological methods.

In this paper we have prezented an optical method based on the determination of optical parameters variations of bacteria during photodynamic inactivation, especially *Staphylococcus aureus* and *Pseudomonas aeruginosa*. By using Kramers-Kronig analysis of the reflectance spectra for two different bacterial culture cells, we can determine the refractive index and absorption coefficient of the bacterial cultures. These optical parameters can be important for management and evaluation of the *in vivo* photodynamic inactivation of bacteria. It is a correlation between the number of bacterial colonies and refractive index of bacterial cultures, which means that based on a curve of calibration it can be, determined the number of colonies from measurements of diffuse reflection.

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