Diffuse reflectance spectroscopy as a new method for the monitoring of the photodynamic inactivation of Pseudomonas aeruginosa

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The large spread of pathogen microorganisms in nature, the existence of numerous sources of infection as well as, the improper and for a long time administration of antibiotics have led to the increasing of the microorganisms resistance realted to these drugs. Among the germs with great resistance to antibiotics are the Staphylococcus and Pseudomonas species. In this context, modern researches in microbiology and related domains (biophysics, biochemistry, medicine, also) were oriented to develop new methods of antimicrobial therapy, more efficient, faster, noninvasive and nontoxic, which do not lead to microbial resistance. One of these noninvasive methods based on the using of light sources and some photosensible substances is photodynamic inactivation of bacteria. Photodynamic inactivation of bacteria depends on some physical variables (the laser parameters, the concentration of photosensitizer in the volume target, etc). The determination of these physical parameters represents an important stage in the experimental studies.

Objective:

The purpose of this paper is the use of the diffuse reflectance spectroscopy as a new method for the determination of the optimal irradiation parameters and the monitoring of the photodynamic inactivation of Pseudomonas aeruginosa

Materials and methods:

The photodynamic inactivation tests use Toluidine Blue (TBO) photosensitizer (C_{TBO} = 8.67x10^{-3}M) and laser system SCL-TR (INOE 2000, Bucharest, Romania, P=15 mW, λ=635 nm).

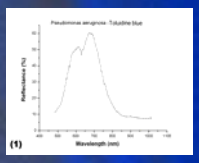
The cultures of *Pseudomonas aeruginosa* had been exposed for 10min, 15min and 20min to laser radiation after 15min from a previous photosensitizer administration.

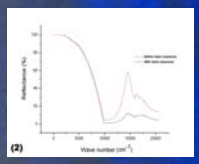
The optical parameters of the bacterial cultures have been determined by diffuse reflectance spectroscopy following the next steps:

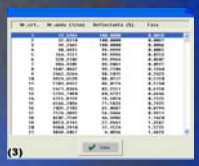
1.the registration and the processing of total diffuse reflection spectra;

2.expanding data of measured reflection in the spectral domains (500 - 0) nm and (1100 - ∞) nm;

3.calculation of phase function φ(ω) from the expanded reflection spectra and the determination of optical constants (ω) and k(ω) from R(ω) and φ(ω).

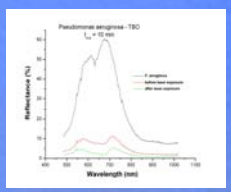


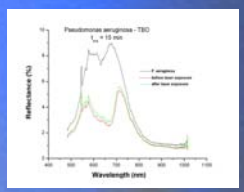


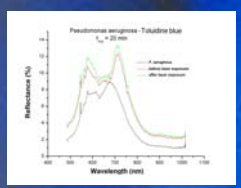


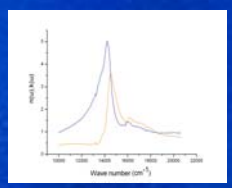
Results:

The reflection spectra of *Pseudomonas aeruginosa* cultures present two main maxima localized at $\lambda_1 \approx 586$ nm and $\lambda_2 \approx 710$ nm. The photodynamic action of the photosensitizer and luminous radiation over bacteria induces the decreasing of the culture reflectance with: ΔR_1 = - 5,2208 % for 10 min exposure time, ΔR_2 = - 2,600 % for 15 min exposure time and ΔR_3 = - 0,6006 % for 20 min exposure time. These demonstrate an increasing of laser radiation absorption which corresponds to a bacterial viability of 95%, 89% and 50% for the above–mentioned exposure times, respectively. A strong dependence between the refractive index n(λ) and the extinction coefficient k(λ) of the medium exists. In the spectral range far from λ_{max} where k(λ) presents a maximum, the n(λ) presents a monotone increase with λ and in the spectral range around the λ_{max}, the n(λ) decreases.









Conclusions:

The contact between *Pseudomonas aeruginosa* and Toluidine Blue for 15 minutes followed by 20 min exposure to laser irradiation produced the lowest bacterial viability (50%) and the lowest variation of the bacterial cultures reflection (ΔR_3= 0,6006%). We can conclude that there is a correlation between diffuse reflection spectra and the bacterial viability. The presented method can be applied method for monitoring the treatment both *in vivo* and *in vitro* studies.