Optical Polarimatic Characterization of Zinc Oxide Nanorods as a Photosensitizer for Photodynamic Therapy

Shamaraz Firdous1*, Amanat Ali1 and Shahzad Anwar1,2

1Biophotonics Lab, National Institute of Lasers and Optronics (NILOP), Pakistan
2National Center for Nanoscience and Technology (NCNST), University of Chinese Academy of Sciences, China

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*Correspondence Address: Shamaraz Firdous, Biophotonics Lab, National Institute of Lasers and Optronics (NILOP), 45650 Islamabad, Pakistan, Email: shamaraz@gmail.com

Abstract

The role of nanoparticles in diagnostic and treatment of cancer and other malignant diseases is gaining much research attention. The zinc oxide nanorods (ZnO NRs) are one of the most studied and promising semiconductor materials for applications in materials and medical sciences. In this study, we present optical properties of ZnO nanorods using Mueller matrix polarimetry to be suitable for its application in photodynamic therapy (PDT). Mueller matrix spectroscopic system capable of acquiring complete polarization information from single nanoparticle to nanostructure in term of transmittance, depolarization, retardance, and polarizance.

The polarization properties measured from 400 to 800nm wavelength of light described decreasing trend of transmission at a wavelength range of 400nm to 530nm. This behavior of light describes Rayleigh scattering in the region and shows ZnO nanorods of relatively smaller diameter to excitation wavelength. Depolarization index decreases from 0.98 to 0.84 on 400nm to 650nm showing bulk scattering of light from randomly distributed ZnO nanorods. Slight change in retardance from 445nm to 550nm shows that light having low frequency and high wavelength passes readily than the high frequency light presenting that ZnO nanorods are strong, compact and composite material. Diattenuation magnitude increases with the increase in wavelength from 400nm to 750nm and changes abruptly at a wavelength range from 750nm to 800nm. This irregular trend indicates that ZnO nanorods are birefringent and anisotropic. These measured polarized properties proved that ZnO NRs are suitable to use as photosensitizer in PDT for diagnostic and treatment of cancer and other malignant diseases.

Keywords: Optical diagnostic; Polarized light; Multiple scattering of light in turbid media; Depolarization; Photodynamic therapy (PDT); Photosensitizer; Zinc oxide nanorods.

Introduction

Nanotechnology and nanoparticles provided great advancement in photodynamic therapy of malignant diseases over the last decades [1-2]. Zinc oxide is a bio-safe and biocompatible material and an attractive candidate for biomedical applications [3-5]. ZnO nanorods (NRs) with high surface area to volume ratio and biocompatibility is a good candidate for efficient photosensitizer (PS) carrier system and at the same time providing intrinsic white light needed to achieve cancer cell necrosis. Drug delivery into the malignant cell is a basic requirement for effectiveness of photosensitizing systems for photodynamic therapy (PDT). For anticancer tumoridal drugs, e.g., 5-aminolevulinic acid (ALA) along with zinc oxide (ZnO) nanoparticles (NPs) used as efficient intracellular photosensitizer carriers [6-8]. Recently, considerable interest has arisen in the optical properties of ZnO nano-material [9-10]. We discuss optical polarization properties of wave propagation through ZnO NRs as a photosensitizer in PDT [11]. This is the fact that polarization methods are giving all possible information of the investigated medium, through light propagation. Scattering of light from turbid medium randomize the state of polarization and wave propagation in such a medium become rapidly depolarized. We have previously reported on depolarization of optical wave propagating through random media for different biomaterials [12-24].

The ZnO nanoparticles, nano flowers and nanorods are used not only also a potential photosensitizer but as shown light extraction efficiency in photonic devices [25]. Due to this, ZnO has a potential for a wide range of applications in UV and intrinsic white light emitting devices. The UV and green emission part of the white light of ZnO may be used to activate some biological process, such as the activation of photosensitizers for photodynamic therapy [26]. Recently photodynamic therapy (PDT) is most widely used for cancerous treatment due to side effects of the traditional treatment strategies. It is a non-invasive,
promising and specially targeting tumoral tissue treatment. In current experimental study, we present optical and non-invasive properties of ZnO nanorods in order to use it as an efficient photosensitizer [27]. Some attractive features of nanomaterials lead to the development of new technologies medical diagnostic procedures and drug delivery system. Nanomaterials used in various biomedical strategies and generate reactive oxygen species (ROS) by interacting with the proteins and enzymes in human cells, which ultimately causes apoptosis or necrosis of malignant cell [28].

The use of ZnO nanoparticles (NPs) for biomedical applications, particularly PDT, relies on the fact that semiconductor nanomaterials could generate reactive oxygen species (ROS) and are promising candidates to become the new generation of photosensitizers. ZnO is an excellent candidate for photosensitizer due to its nontoxicity and ability of biodegrade with high thermal and chemical stabilities [29]. Optical polarization properties of the scattered light are crucial for fundamental understanding of the aforementioned effects because polarization plays an important role in the light-matter interactions effects. Moreover, the polarization information should also exploited to develop polarization-controlled novel schemes for contrast enhancement in biomedical imaging using Mueller matrix model. Mueller matrix is a 4×4 matrix representing the transfer function of any optical system in its interaction with polarized light and all the medium polarization properties are characteristically encoded in its various elements.

The recorded Mueller matrix analyzed to extract or quantify the intrinsic polarization properties of the medium. The resulting polarization properties, transmittance, depolarization, retardance, and polarizance, potentially utilized for quantitative analysis / interpretation of biomaterials [30,31].

Material and Methods

We have purchased the reagents including zinc acetate dehydrate and sodium hydroxide from Sigma-Aldrich Company (UK) and synthesized ZnO nanorods (ZnO-NRs) through hydrothermal technique. Precursor used for ZnO nanorods preparation are zinc acetate dehydrate Zn (CH₃COO)₂•2H₂O (98%) and sodium hydroxide NaOH (98%) [32,33]. The Mueller matrix polarimetric system (AxioScan, Axometrics, Inc., U.S.A.) consist on polarization state generator (PSG), polarization state analyzer (PSA), and spectrally resolved signal detection unit. Collimated white light from a mercury lamp used as an excitation source and is passed through the PSG unit for generating the input polarization states. The PSG unit consists of a horizontally oriented fixed linear polarizer P₁ and a rotatable quarter wave plate (QWP₁) mounted on a computer controlled rotational mount. The PSG-emerging light focused to the sample and the sample and scattered light collected by the PSA unit for the analysis of the polarization state of light and relayed for spectrally resolved signal detection. The PSA unit comprises of the same polarization components with a fixed linear polarizer (P₂) and a computer controlled rotating quarter wave plate (QWP₂) as shown in Figure 1 [34].

Result and Discussion

In our research, we used Mueller matrix polarimetric model for the optical characterization of Zinc oxide metallic nanorods to use in photodynamic therapy (PDT). For the synthesize of photosensitizer (ZnO nanoparticles), we used stock solutions of Zn (CH₃COO)₂•2H₂O (0.1M) s prepared in 50ml methanol under stirring. To this stock solution, 25ml of NaOH (varying from 0.2M to 0.5M) solution prepared in methanol added under continuous stirring in order to get the pH value of reactants between 8 and 11. These solutions was transferred into teflon lined sealed stainless steel autoclaves and maintained at various temperature in the range of 100 – 200 °C for 6 and 12h under autogenous pressure. It then allowed cooling naturally to room temperature. After the reaction was complete, the resulting white solid products washed with methanol, filtered and then dried in air in a laboratory oven at 60 °C [35].

The synthesized samples characterized for their structure with optical polarized light Mueller matrix system. The demonstrated novel ability to record full polarization information over a broad wavelength range and to quantify the
intrinsic polarimetry characteristics via Mueller matrix analysis should lead to a novel route towards quantitative understanding, analysis or interpretation of a number of effects and may prove useful towards development of an efficient photosensitizer for photodynamic therapy [36]. The following polarization properties were experimentally measured to characterize ZnO NRs to be suitable used as photosensitizer in PDT.

**Polarized light transmittance of ZnO nanorods**

The transmittance behavior of radiations having wavelength in the range of 400-800nm indicates that ZnO nanorods absorb radiations of longer wavelength (red light) and emit the radiations of shorter wavelength (blue light). Rayleigh scattering is dominant for the particles smaller than an excitation wavelength of light 400.

The decreasing trend of transmission at a wavelength range of 400nm to 530nm provide Rayleigh scattering in this region and ZnO nanorods of relatively smaller diameter are present as shown in Figure 2. It is shown that there is slight increase in transmission of light with increase in wavelength from 530nm to 800nm showing Mie scattering of light is high in this wavelength range and ZnO nanorods of larger size are randomly distributed.

![Figure 2: The ZnO Nanorods transmittance plot at the wavelength of 400-800nm of incident light. The Mueller matrix polarized light transmission indicates the optical characterization of the structure of the synthesized material.](image)

**Polarized Light Depolarization Index of ZnO Nanorods**

The depolarization of light provides the mixing of a polarized light coupled into unpolarized light. The depolarization exists only when the incident light is polarized and the degree of polarization of emission light is less than excitation. The surface roughness and bulk scattering value of depolarization index lies in the range from 0 to 1, corresponds to the unpolarized light compared to polarized light.

![Figure 3: The depolarization index plot of ZnO nanorods. The Mueller matrix polarized light depolarization with wavelength of light indicates the optical characterization of the structure of the synthesized material.](image)
The blue line in Figure 3 shows depolarization of light for actual trend obtained and red line represents the average trend. When the wavelength increases from 400nm to 650nm, depolarization index decreases from 0.98 to 0.84 indicating that depolarization is occurring due to bulk scattering of light from randomly distributed ZnO nanorods. This scattering is Rayleigh scattering pointing to the smaller size of nanorods. There is a slight increase in depolarization index value from 0.84 to 0.86 for light wavelength of 650nm to 800nm. The trend represent that scattering is saturated at 650nm wavelength of light.

**Polarized Light Retardance Magnitude of ZnO Nanorods**

The polarization retarder introduces a polarization dependent phase change of light between its eigen polarizations in term of retardance. One eigen polarizations shows the maximum transmitted phase and the other shows the minimum transmitted phase, in between these two remaining polarization states show intermediate phase change.

The retardance of ZnO nanorods is represented in Figure 4, actual results and average of these results represented by blue and red line respectively. Retardance decreases with the increase in wavelength from 445nm to 550nm and after that, there is a slight change in retardance with the increase in wavelength up to 800nm. This trend represents light having low frequency and high wavelength passes readily than the higher frequency light providing ZnO nanorods, strong, compact and composite material.

**Polarized Light Diattenuation Magnitude of ZnO Nanorods**

The diattenuation arises when the intensity transmission of the exiting beam is a function of the polarization state of the incident beam. For incident state intensity, transmittance is maximum and minimum for orthogonal state. In Figure 5, we observed that diattenuation magnitude increases slightly with the increase in wavelength from 400nm to 750nm and changes abruptly at a wavelength range from 750nm to 800nm. This irregular trend indicates that ZnO nanorods are birefringent and anistropic 400.
Polarized Light Polarizance Magnitude of ZnO Nanorods

The polarizance is the degree of polarization of the exiting beam when the unpolarized light is incident. Polarizance is just reverse of the depolarization during which unpolarized light transformed into the polarized light. It can be seen in Figure 6, that there is a slight increase in polarizance with the increase in wavelength from 400nm to 800nm which indicates that ZnO-NRs does not transform the incident unpolarized light to the polarized light due to bulk scattering with the small size particles.

Figure 6: The polarizance magnitude plot of ZnO nanorods. The Mueller matrix polarized light polarizance with respect to incident light wavelength indicates the optical characterization of the structure of the synthesized material.

Polarized Light Intensity Transmittance model of ZnO Nanorods

The optical properties of ZnO nanorods directly investigated by intensity transmittance model with 64 elements of polarization measurements at various wavelengths of light as represented in Figure 7. This model reveals information about the diattenuation matrix, retardance matrix, and depolarization matrix. First element of Muller matrix represents direct intensity transmittance. Transmission intensity determined by the first row of the Muller matrix. If diagonal elements of the matrix have same value (intensity), then it represents the retardance Muller matrix and diagonal elements of the depolarization matrix. In this way we have easily determine optical properties by interpreting the Muller matrix elements representing in this model.

Figure 7: The intensity transmittance Mueller matrix model for ZnO NRs at 400-800nm wavelength. The Mueller matrix polarized light model indicates the optical characterization of the structure of the synthesized material.
The considerably low magnitudes of the diagonal elements imply overall strong depolarizing nature of the scattered light. Non-zero intensities of the elements in the first row and the first column on the other hand, is a manifestation of the linear diattenuation effect. Strong signature of the linear retardance effect is also evident from significant intensities of the off-diagonal elements. In our previous study, Fakhar et al. [6] introduced the concept of Drug delivery into the malignant cell for effectiveness of photosensitizing systems for photodynamic therapy. Apoptotic effect of tumouricidal drugs (ALA and Photofrin®) is studied in HepG2 (Hepatocellular carcinoma) in the presence and absence of ZnO NPs using confocal microscopy as well as Neutral Red Assay (NRA). In dark, ZnO NPs conjugated with ALA or Photofrin® has been found to have a remarkable fluorescence in Hepatocellular carcinoma (HepG2) cells [37]. This fact illustrates the great potential of ZnO NPs as biomarker in relevant clinical and biomedical applications.

In another study by Ravendra K Pandey et. al. [38] covers many aspects of PDT and nanotechnology. In his research, he developed multifunctional theranostic nanoparticles, which can carry out both tumor imaging using PET, MRI, and fluorescence and at the same time can mediate tumor therapy via PDT. He also develops models for photosynthetic reaction centers and deeply explores the chemistry of porphyrin-based compounds related to chlorines and bacteriochlorin. Most of the photosensitizers and tumor-imaging agents developed in his group are derived from naturally occurring chlorophyll-a, bacteriochlorophyl1 a, and several more are in various stages of preclinical and toxicological studies [37,38]. The Paras N Parsad et al. [39] presented his research for the preparation, processing, and theoretical modeling of photonic materials for PDT and interested in investigating multifunctional nanostructured materials that can do both therapy and fluorescence imaging and in particular in multiphoton processes and their applications to PDT. Recent results are consistent to our previous findings and other data available in literature.

Conclusion

The ZnO nanoparticles characterized and used as a photosensitizer along with ALA and Photofrin in HepG2 cells as described in our previous research. The Mueller matrix polarimetric model successfully presented the optical characterization of ZnO nanorods in term of polarization, depolarization index, retardance, diattenuation and polarization may be helpful for structural changes and particles nature study of ZnO nanorods. The experimental results conclude that the ZnO nanorods are strong, highly compatible and dense scattering material suitable candidate for PDT and as a kind of low-cost, low toxic and versatile material, have shown to have a promising future in biological applications. However, there are still challenges in front of researchers to improve or change ZnO fluorescence, ZnO bio imaging on the multiple target cells and live animals. Antibacterial and anticancer performances of ZnO NRs have shown a bright future, but they all require plenty of work before any practical applications.

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