

Application of laser confocal fluorescence spectroscopy in studies of spatial distribution of porphyrin molecules on plasma deposited polymer films

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Laser confocal microscopy combined with spatially-resolved fluorescence spectroscopy were used for the analysis of surface distribution and state of di(*p*-aminophenyl)etioporphyrin (DAPEP) and tetra(*p*-aminophenyl)porphyrin (TAPP) molecules deposited on thin (7–15 μm) treated by 0.5 M KCl solution and activated by air non-equilibrium plasma at the normal atmospheric pressure. Confocal microscopy data show that after treatment the polymer surface becomes inhomogeneous, and porphyrin moieties are randomly distributed on both film surfaces with a penetration depth of ~1 μm. On the basis of spatially resolved fluorescence measurements it has been found that fluorescence spectra of individual spots correspond to monomeric species. It means that spatially closed few porphyrin molecules in the spot are not aggregated and do not interact significantly.

Keywords: porphyrins, polypropylene films, plasma treatment, laser confocal microscopy, spatially resolved fluorescence spectroscopy.

Introduction

It is well known that polymers are widely used for a broad field of applications [1]. Ion irradiation within few eV to GeV is an efficient tool to modify the properties of polymers (wettability, optical characteristics, adhesion, etc. [2]). Polypropylene (PP) being one of the most inert polymers with low sorption properties is widely used in medico-biological applications. On the other hand, tetrapyrrolic compounds being fixed on synthetic polymer carries by various methods or being polymers themselves [3, 4] are widely used both as model compounds of natural polymer-bonded tetrapyrroles (chlorophyll, haemoglobin, cytochrome) as well as membranes and sensors. Noteworthy, the fixation of monomeric porphyrins, chlorines or bacteriochlorins on the inert PP film may be considered for possible applications in photodynamic therapy [5] and blood cleaning [3] as well as for the development of optical limiters.

In this contribution, we present the results showing the possibilities of the modern experimental methods such as laser confocal microscopy and spatially-resolved fluorescence spectroscopy) for the study of the interaction of biologically active molecules with polymeric surfaces. Specifically, these methods open the possibility to control the distribution of active organic component on the film surface as well as to discriminate the nature (monomeric or aggregated forms) of adsorbed molecular species.

Results and Discussion

Basic results were obtained for di(*p*-aminophenyl)etioporphyrin (DAPEP) and tetra(*p*-aminophenyl) porphyrin (TAPP) molecules (Fig. 1) upon their interaction with the surface of thin (7.2–15 μm) polypropylene (PP) films activated by air non-equilibrium plasma at normal atmospheric pressure. The experimental setup for the atmospheric pressure plasma deposition with liquid electrolyte and the preparation of “PP-porphyrin” samples was described in [6]. It was supposedly believed that side active amino groups may be responsible for the non-covalent attachment of porphyrin macrocycles to the PP film surface. Samples of commercial isotactic PP films with the thickness of 7–15 μm have been cleaned by ethanol and distilled water and dried under vacuum before plasma treatment.

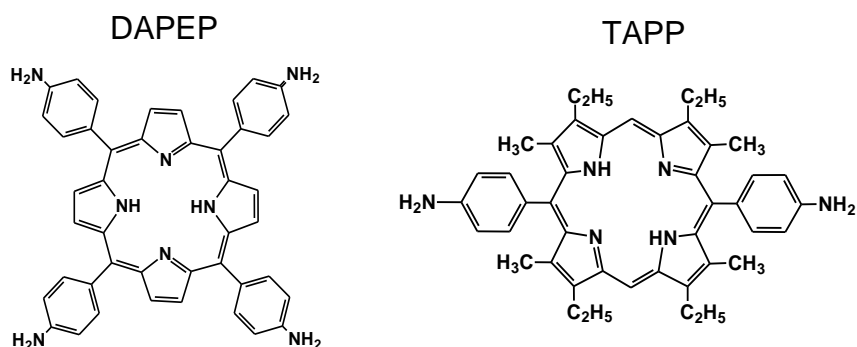


Fig. 1. – Structures of di-(*p*-aminophenyl)etioporphyrin (DAPEP) and tetra-(*p*-aminophenyl) porphyrin (TAPP) molecules.

The comparative results are discussed for the following samples: (1A) is a control sample with a non-treated film, (2A) is the film treated by O₂-plasma in zone of positive range of charge + DAPEP, (3A) is the film treated by O₂-plasma in after-emission zone + DAPEP, (1B) is the film treated by O₂-plasma in zone of positive range of charge, (2B) is the film treated by O₂-plasma in after-emission zone, (3B) is the film treated by solution plasma, (4B) is the non-treated film + TAPP, (5B) is the non-treated film + DAPEP, (6B) is the film treated by O₂-plasma in after-emission zone + DAPP.

The commercial laser scanning microscope LSM 510 (Release 2.8, Carl Zeiss Germany, the spatial resolution ≥ 200 nm) has been used in order to measure the thickness of PP films as well to analyse the porphyrin distribution from both sides of the films. To measure the thickness of the films, some x-z-cuts through the films have been recorded in reflection and fluorescence modes, for each film cut images have been obtained with different magnification (Fig. 2). Typically, the films were recorded in reflection mode (excitation by 514.5 nm line of the argon ion laser), because the resolution is much better than in fluorescence regime recording.

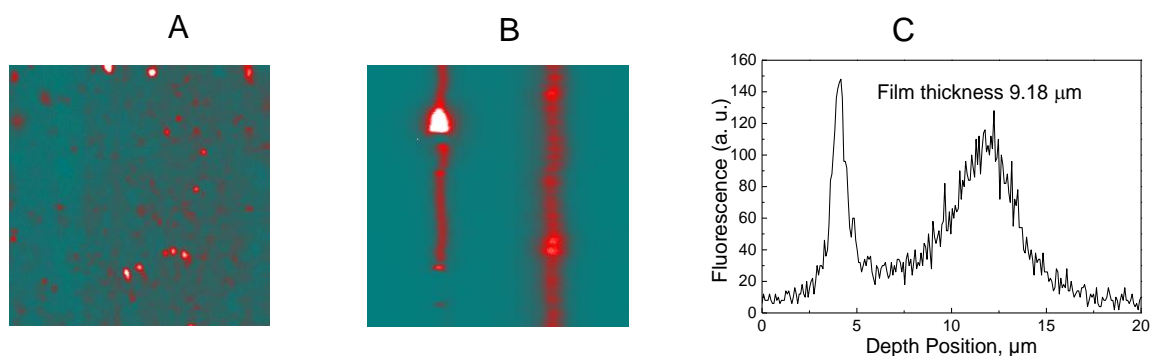


Fig. 2. – Lateral fluorescence image (A), cut of the fluorescence image (B) and depth profile of the fluorescence image cut (C) for PP film ($20 \times 20 \mu\text{m}^2$) treated by O₂-plasma in after-emission zone + DAPEP.

In fluorescence mode (Fig. 2, C) the left peak corresponds to the upper side of PP film, the right one corresponds to the lower side. The analysis of fluorescence profiles of cut images shows rather strong differences in the distributions of the porphyrins on film surfaces treated by various ways. From these findings it may be concluded that after plasma treatment the polymer surface becomes non-homogeneous, and porphyrin moieties are seen to be randomly distributed on both film surfaces with a penetration depth of $\sim 1 \mu\text{m}$. The next step is to clearly recognize the state of the molecules on the PP surface (monomeric or aggregated, at least).

Correspondingly, we would like to come to the fluorescence part of the work (the spatially-resolved emission of individual objects which seems to be more interesting. One

primary motivation for the studying the spatially-resolved emission of individual objects in solids, liquids, proteins or on interfaces and surfaces is due to the possibilities to elucidate both the local environment influence (heterogeneous by nature) and the individual object state and properties, so that the behavior of individuals may not be fully represented by the standard (large number N) ensemble average. At present, conventional confocal microscope technique (home-built presumably) permits to carry out steady-state and kinetic fluorescent measurements in a wide temperature range and has now broad interdisciplinary impact, ranging from physical and analytical chemistry to biophysics, medicine, material science and nanotechnology.

Spatially resolved fluorescence measurements have been carried out by means of a home-built confocal microscope described in details in [7]. The samples mounted on a closed-loop xyz-piezo translation stage (Piezosystems Jena) have been excited by 482 nm line of a Krypton ion laser or the 514.5 nm line of an Argon ion laser. The light was coupled into the set-up via an optical fiber and focused onto the sample by an objective (Carl Zeiss Jena, 100 \times magnification, aperture of 0.9) and then detected by a liquid-nitrogen-cooled CCD (Princeton Instruments) with a time resolution up to 100 ms/frame and high spatial resolution. In order to verify the existence of porphyrins in bright spots of PP film surface we have excited these bright spots by the narrow laser beam and detected the spectral distribution of this emission using home-built confocal microscope. In Fig. 3, A one examined spot is shown by a white arrow while the other arrow shows PP film surface without porphyrin in order to detect the background emission.

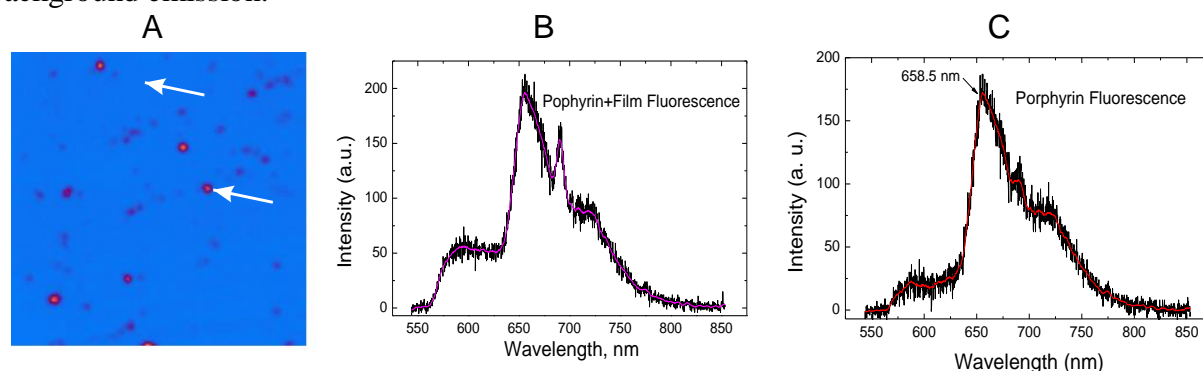


Fig. 3. – Emission image of PP film ($20 \times 20 \mu\text{m}^2$) treated by O_2 -plasma in after-emission zone + TAPP (A) and fluorescence spectra of the PP film spot with porphyrin (B), the spectrum shown in part (C) is the fluorescence of the attached porphyrin molecules after subtracted admixture of the PP film emission. $\lambda_{\text{exc}} = 407 \text{ nm}$, power density $N = 25 \mu\text{W}/\mu\text{m}^2$, 295 K.

The comparison of fluorescence profiles and the maximum positions obtained for the spots with subtraction of PP film emission (Fig. 3C) with those detected in ensemble measurements for monomeric TAPP molecules in toluene shows a good coincidence. Thus, it may be concluded that for bright spots on PP film surface the emission originates from monomeric TAPP molecules. The same results have been obtained in numerous experiments with both TAPP and DAPEP molecules absorbed on PP films treated in various plasma conditions. In this respect, it should be mentioned that the lateral resolution of the confocal setup does not exceed $\Delta l \approx 235 \text{ nm}$ and a vertical resolution is of 970 nm. So, spots may contain few or even more porphyrin molecules disposed closely to each other. Thus the possibility of porphyrin aggregation is not excluded. For TAPP molecules on PP film surfaces, ensemble average fluorescence excitation spectra in UV-VIS region (relative intensities of the bands) coincide with the same distribution measured for monomeric TAPP molecules in toluene. The decrease of stokes shift down to $\sim 70 \text{ cm}^{-1}$ in comparison with 360 cm^{-1} in toluene solution may be caused by a more rigid fixation of TAPP molecules on the PP film surface. Correspondingly,

in the spot the fluorescing species of TAAP molecules as monomeric ones may be taken as proved. In addition, spontaneous porphyrin aggregates and oligomeric/polymeric porphyrins are characterised by low fluorescence efficiencies. Accordingly, TAAP spontaneous aggregates are hardly detected by emission with respect to monomers in one spot. But in the case of the considerable existence of aggregated TAPP species together with monomeric form in one spot at relatively small distances, the non-radiative resonant energy transfer “monomer→aggregate” should take place, thus leading to the monomer fluorescence decay strong shortening. Nevertheless, our preliminary time-correlated single photon counting measurements have shown that fluorescence decay of TAPP in spots (three have been analysed) is of $8.7\div 9.3$ ns that is typical for monomeric porphyrins of various types in liquid solvents in the presence of dissolved oxygen.

Conclusions

On the basis of laser confocal microscopy combined with spatially-resolved fluorescence spectroscopy methods and comparison with ensemble averaged measurements it has been elucidated that fluorescence spectra of individual bright spots on film surfaces correspond to porphyrin monomeric species. In this case, spatially closed few porphyrin molecules in the spot do not form aggregates. PP films with low surface energy and low sorption properties being surfacely activated by porphyrins (in monomeric form known as effective sensitizers of singlet oxygen $^1\Delta_g$) may be used in photodynamic therapy and blood cleaning.

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References

1. Goddard J. M., Hotchkiss J. H. Polymer surface modification for the attachment of bioactive compounds. *Progress in Polymer Science*. 2007. Vol. 32, № 7. P. 698–725.
2. Rueck D. M. *Nuclear Instruments and Methods in Physics Research. Section B: Beam Interactions with Materials and Atoms*. 2000. Vol. 166–167. P. 602–609.
3. Koifman O. I., Ageeva T. A. *Porphyrinopolymers*. Phys.-Math. Lit. Press. Moscow, 2006.
4. Zenkevich E. I., von Borczyskowski C. Photoinduced relaxation processes in self-assembled nanostructures: multiporphyrin complexes and composites “CdSe/ZnS quantum dot-porphyrin”. In: “Multiporphyrin Arrays: Fundamentals and Applications”. Ed. D. Kim. Pan Stanford Publishing Pte. Ltd., Singapore, 2012. Chapter 5, P. 217–288.
5. Zenkevich E., von Borczyskowski C. Surface Photochemistry of Quantum Dot-Porphyrin Nanoassemblies for Singlet Oxygen Generation. In “Photoinduced Processes at Surfaces and in Nanomaterials”. Ed. D. Kilin. Washington, ACS Symposium Series, 2015. Vol. 1196. P. 235–272.
6. Golubchikov O. A., Ageeva T. A., Titov V. A., Vershinina I. A., Shikova T. G., Semeikin A. S., Maksimov A. I., Zenkevich E. I. Method of the surface modification for propylene material. The Patent of Russian Federation № 2223982, Invention Bulletin № 5 (20.02.2004).
7. Cichos F., Martin J., von Borczyskowski, C. Emission intermittency in silicon nanocrystals. *Phys. Rev. B*. 2004. Vol. 70. P. 115314.