



Antimicrobial polyhydroxybutyrate submicron fiber mat loaded with zinc phthalocyanine derivative

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Abstract The aim of this work was to prepare a new biodegradable polyhydroxybutyrate (PHB) submicron fiber mat loaded with phthalocyanine (Ptc) derivative by centrifugal spinning technology, an alternative approach to fabricate nanofibers or microfibers from solutions at high speed and low cost. The results proved a significant production of singlet oxygen by the prepared photocatalytic fiber mat. In contrast with the results of other research groups published before, we have found a very significant antibacterial activity of a neutral zinc Ptc derivative, entrapped in PHB submicron fiber matrix, against Gram-negative *Escherichia coli* bacteria under visible light irradiation and even in the dark. On the contrary, the antibacterial effect of the photocatalytic fiber mat against Gram-positive *Enterococcus faecalis* was only moderate under visible light irradiation and no effect was observed in the dark.

Keywords centrifugal spinning, polyhydroxybutyrate, photocatalytic nanofiber, zinc phthalocyanine, antibacterial

1. Introduction

Phthalocyanines (Pcs), tetrabenzo-fused 5,10,15,20-tetraazaporphyrins, were initially described in 1930. Since that time many applications have appeared, those based on their extensive and distinctive color properties have dominated. Because of their stability and light absorption in characteristic regions of the visible part of spectrum, phthalocyanines have traditionally been used as pigments and dyes. Thereafter, other important applications have appeared based on their ability to generate an active species upon light absorption. These photoinduced events may occur due to the ability of some phthalocyanines to excite ground-state molecular oxygen ($^3\text{O}_2$) to highly active singlet oxygen ($^1\text{O}_2$) [1]. Phthalocyanines belong to the group of photosensitizers together with porphyrins or methylene blue [2]. Their maximum light absorption occurs in the visible portion of the electromagnetic spectrum, which constitutes a larger portion of the spectrum and thus more available than the ultra-violet portion required by other sensitizers [3]. Introducing diamagnetic metals such as Zn, Si, Al, Ga and In into the cavity of the Pc ring results in enhanced triplet quantum yields and lifetimes and high singlet oxygen quantum yields [4]. However, it must be noted that, only phthalocyanines containing transition metal ions with a closed valence shell such as Al^{3+} , Si^{4+} or Zn^{2+} are efficient photocatalysts in the production of $^1\text{O}_2$. Those containing metal ions with partially filled d-orbitals such as Co^{2+} and Cu^{2+} tend towards rapid extinction of their excited triplet states [1]. Metallophthalocyanines (MPcs) have attracted the attention of many researchers due to their numerous applications including in non-linear optics, electrochemical sensors, photodynamic therapy (PDT) of cancer, dye-sensitized solar cells, as well as photocatalysis. MPcs possess properties such as relatively high extinction coefficients, excellent visible/near infrared absorption, high



chemical and thermal stability and have the ability to generate singlet oxygen which also makes them ideal for photocatalytic applications [5]. The chemical structure of phthalocyanines has been modified and derivatized in many different ways. These changes can strongly modulate the photophysical properties [6].

The use of symmetrically substituted Pcs as photosensitizers is well established, however these complexes show low substrate binding selectivities. It has also been reported that decreasing the symmetry of phthalocyanines improves the singlet oxygen quantum values [7].

Applications in industry, medicine, and biology have shown the great importance of phthalocyanine molecules [8]. Singlet oxygen and other reactive oxygen species have strong biocidal effect on algae, bacteria, fungi, yeasts and cyanobacteria [9-14]. Photocatalysis has been recognized as a promising technique for water and air purification and disinfection and has since been studied extensively as an alternative to currently used technologies, such as chlorination, ozonation and adsorption on active carbon [15]. MPcs have attracted interest for visible light photocatalytic degradation of toxic organic pollutants [16]. Singlet oxygen is an extremely effective agent for complete oxidation of common pollutants of water including chlorinated phenols [1].

Catalytic activation of peroxides by MPcs to generate radicals for oxidative degradation of different resistant organic pollutants has received considerable and increasing attention in environmental catalysis field, too [1,17-21,37].

Increasing antimicrobial resistance requires the development of novel materials and approaches for treatment of various infections. Utilization of photodynamic therapy represents an advanced alternative to antibiotics and metal-based agents. Such materials, including MPcs, can be potentially used in antibacterial therapy of chronic wounds, infections of diabetic ulcers, and burns, as well as rapidly spreading and intractable soft-tissue infections caused by resistant bacteria [22] and cancer therapy [23].

MPcs are perspective materials in the field of organic synthesis, too. Using singlet oxygen to synthesize natural products and drugs has been reviewed recently [24]. Even though singlet oxygen is a short-lived metastable excited state of molecular oxygen, it is a practical reagent for compound oxidation and can form carbon-oxygen and heteroatom-oxygen bonds. Progress has been made regarding $^1\text{O}_2$ in the organic synthesis of complex targets. Synthesis of natural products is often modeled on possible $^1\text{O}_2$ biosynthetic routes. Thus, biomimetic $^1\text{O}_2$ reactions that use alkene and diene precursors are common; however, fewer examples are known with polyenes, di- and polysulfides, and amines due to their facile physical quenching of singlet oxygen ($^1\text{O}_2 \rightarrow ^3\text{O}_2$).

The catalytic performance of homogeneous MPC catalyst is limited by the aggregation tendency of phthalocyanine molecules, which would negatively produce catalytically inactive dimers [25]. What is more, the catalyst may also be deactivated by the oxidative self-destruction of MPC molecules during the catalytic oxidation [17]. To solve these problems, numerous support materials have been employed to immobilize MPC molecules to prepare the hetero-geneous catalysts [21]. The immobilization of nano-sized photocatalysts can eliminate costly and impractical post-treatment recovery of spent photocatalysts in large scale operations. Factors such as high durability, ease of availability, low density, chemical inertness and mechanical stability are primary factors responsible for the selection of suitable supports for catalysts [26]. Some employed immobilisation supports include ZnO microparticles [27], nanoporous ZnO films [7], magnetic nanoparticles [5,28], gold nanoparticles [29,30], silver nanoparticles [31,32], graphene [33,34], carbon nanotubes [35,36], chitosan microspheres [37], beads [31], and pellets [38], or Al_2O_3 solid matrix [1]. Tome, et al. [39] synthesized new poly-s-lysine-porphyrin conjugates and showed that the cationic conjugates are able to photosensitize the efficient inactivation of both Gram-positive and Gram-negative bacteria. Modification of phthalocyanines through metal nanoparticle doping is increasingly being considered for maximizing their singlet oxygen generation, hence their photocatalytic efficiency [29].

Among Pcs and MPcs supports, fibrous materials have been considered as ideal candidates exhibiting particular advantages, such as suitable mechanical and chemical stability, low cost and flexibility in modeling different shapes [21]. Pcs or MPcs can be embedded in polymeric fibers being spun directly by simple addition into the polymeric solution or with a covalent bond so that they could not escape spontaneously from the filtration materials [40]. The photosensitizers can also be anchored to the fiber surface, usually by a covalent bond, as such, or in a form of a complex or a conjugate prepared earlier. Different polymers have already been used to immobilize Pcs or MPcs in or on the fibers, such as polystyrene [4,5,13,15,27,29,32,41-44], polyacrylonitrile



[18,20], polyamide 6 [13,28], activated carbon fibers [17,19], polyurethane [13,40], chitosan [22,38], polycaprolactone [13], polysulfone [3], polymethyl methacrylate [30], or conductive polyvinylpyrrolidone [42]. Polystyrene (PS) was chosen by many research groups as a support material because of its extensive aromatic system which allows for electronic interactions between the aromatic systems of the phthalocyanine and the polymer. Hybrid electrospun fibers provide great potential as active photocatalysts for oxidizing organic pollutants using singlet oxygen as an oxidant [29]. Binuclear MPc supported on treated silk fibres has been successfully tested as a novel air-purifying material [45]. A new approach to enable the metal ions to interact with MPc through fibrous support, constructing a novel bimetallic photocatalyst via simultaneously immobilizing iron(II) phthalocyanine (FePc) and Cu(II) onto the surface of amidoximated polyacrylonitrile (PAN) fiber, has been described recently [21].

Inorganic semiconductors with appropriate structures have been widely reported as photoinduced oxidative catalysts, covering the advantages like high oxidizing capacity, non-toxicity and remarkable chemical stability. However, they suffer from low catalytic activity under visible light irradiation due to their intrinsic wide band gap, and the difficulties in nano-scaled substrate recycling [21]. Machado, et al. [46] reported the characterization of composites prepared by the association between zinc phthalocyanine and titanium dioxide. These composites are better photocatalysts for wastewater decontamination mediated by solar radiation than pure TiO₂. PCs sensitized titanium dioxide catalyst is able to achieve the degradation of many organic pollutants by visible light as energy and O₂ in the air as the oxidant under ambient conditions. Sensitization is an economic and technological option to improve photo-activity of TiO₂ in visible range of electromagnetic spectrum [47].

The aim of this work was to prepare a new biodegradable polyhydroxybutyrate submicron fiber mat loaded with a zinc phthalocyanine derivative by centrifugal spinning technology, an alternative approach to fabricate nanofibers or microfibers from solutions at high speed and low cost.

2. Materials and Methods

2.1. Synthesis of the zinc phthalocyanine derivative

The ZnPTC-OA zinc phthalocyanine derivative was synthesized in the Centre of Organic Chemistry (Rybitví, Czech Republic). The polyhydroxybutyrate polymer BIOMER was purchased from Biomer company (Germany). All other chemicals were purchased at Merck, Czech Republic.

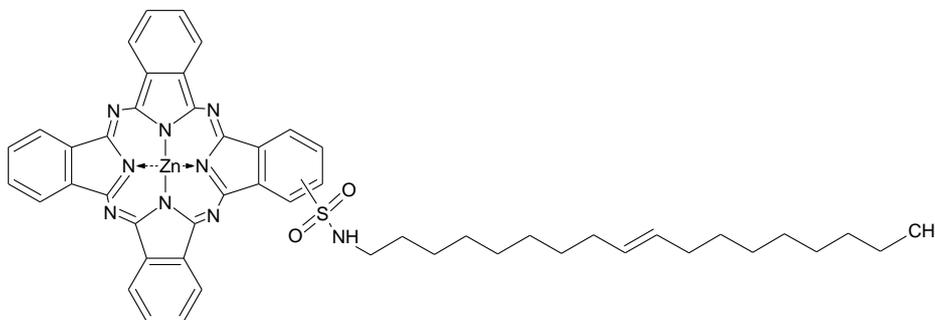


Figure 1: Structure of the Zn phthalocyanine derivative

The singlet oxygen (¹O₂) quantum yield of this molecule $\phi = 0,59$, was determined by the method based on using the singlet oxygen chemical quencher 9,10-dimethylantracene (DMA) in dimethylformamide, described earlier [48].

2.2. Preparation of the PHB submicron fiber mat by the centrifugal spinning technology

The NANOCENT demonstrator for the centrifugal spinning technology was used to produce the polyhydroxybutyrate (PHB) submicron fiber mats. The demonstrator is based on a new method for preparation of submicron fibers and can be adjusted to produce various types of fiber membranes and 3D constructs for use in wide spectrum of industry applications. The submicron fiber production is based on improved centrifugal technology [49], using a fiber-forming conical hollow disc, composed from lower and upper parts forming an inner space with an outlet gap in-between them, connected with a hollow shaft and rotating around the axis at 1500 - 5000 rpm. Via openings a polymeric solution is sprayed from an inner space of the hollow shaft to the



inner space of the rotating disc. The fibers are formed by centrifugal force at the edge of the outlet gap and collected in form a homogenous mats on a cylindrical collector positioned beside the fiber-forming disc and rotating at 1000 - 12000 rpm (see Fig. 2,3).

For these experiments, 2% (w/w) solution of the PHB polymer in chloroform was prepared. PHB was dissolved at 70 °C under continual shaking for 48 hours. The ZnPTC-OA sample was added to the PHB chloroform solution in a form of 10% (w/w) solution in tetrahydrofuran to reach 0.1% (w/w) final concentration of the phthalocyanine derivative in the resulting solution.

Pure white PHB and the composite greenish-blue PHB-ZnPTC-OA submicron fiber mats were prepared with the NANOCENT demonstrator under conditions summarized in the following Table 1.

Table 1: Parameters of the fiber forming process

Rotational speed of the fiber-forming disk	3500 rpm
Flow rate of the solution	10 ml/min
Air flow rate	65 m ³ /h
Input air temperature	22 °C
Input air relative humidity	31,00%
Rotational speed of the fiber collector	5000 rpm



Figure 2: Preparation of the PHB submicron fiber mat by the centrifugal spinning technology and a rotating fiber collector



Figure 3: Preparation of the PHB submicron fiber mat and cobweb like structures, loaded with the Zn phthalocyanine derivative, by the centrifugal spinning technology and a rotating fiber collector

2.3. Characterisation of the fiber mats by Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was used to analyze the particle sizes and morphologies. The samples of the fibers were fixed with a double-faced adhesive tape to the holders and evaluated in a Phenom G2 scanning electron microscope (Phenom-World BV, Eindhoven, Netherlands).



2.4. Singlet oxygen production quantification by the 1,3-diphenylisobenzofuran method in hexan

Indicator 1,3-diphenylisobenzofuran (DPIBF) reacts with intermediate singlet oxygen $^1\text{O}_2$, forming an unstable peroxide that decomposes into colorless 1,2-dibenzoylbenzene [50], under the following scheme, see Fig. 4.

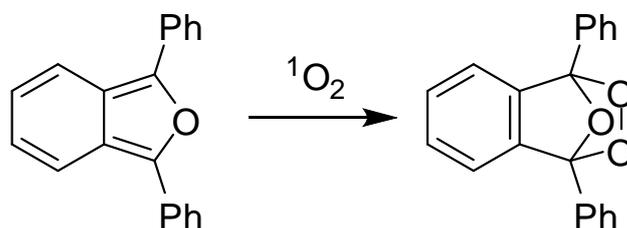


Figure 4: Scheme of reaction of DPIBF with $^1\text{O}_2$

The method is selective for determination of singlet oxygen $^1\text{O}_2$ production. No other reactive oxygen forms don't affect the result. Hexan was chosen as a nonpolar solvent because it can dissolve the DPIBF indicator, but PHB and the ZnPTC-OA phthalocyanine derivative are insoluble in it. Moreover, the molar ratio of the absorbed oxygen is even higher than in water [51].

Squares of size 8x8 mm were cut from the fiber mats and placed at the bottom of a spectrophotometer cuvette. Each sample was measured in parallel. The square was weighed accurately on analytical balances. Then, 2 ml of hexan were pipetted to the cuvette and such amount of the DPIBF indicator ($c \sim 2\text{g/l}$) was added to reach absorbance $\lambda = 417\text{ nm}$ in the range 0.7 – 0.8. The sample in the cuvette was subsequently illuminated by a laser with a focused beam of light at $\lambda = 661\text{ nm}$ and power 40 mW. The reaction rate was followed by measurement of light absorbance at $\lambda = 417\text{ nm}$, when absorbance decrease occurs because of the reaction of the indicator with singlet oxygen $^1\text{O}_2$ in dependence on the light energy emitted from the laser. From the slopes $k\ (\text{J}^{-1})$, which are proportional to the reaction rate, the indicator degradation half-time values $\tau_{1/2}\ (\text{s})$ were calculated for each of the samples. The $k\ (\text{J}^{-1})$ and $\tau_{1/2}\ (\text{s})$ values were recalculated for unified weight 1.5 mg.

2.5. Spectrophotometric determination of the ZnPTC-OA photosensitizer concentrations in the PHB fiber mats

To verify homogeneity of the ZnPTC-OA photosensitizer concentration in the PHB fiber mats, a simple spectrophotometric method was developed. 10 mg samples of the fiber mats were extracted with 80 ml tetrahydrofuran 15 min under continuous agitation at laboratory temperature. Absorbance of the extracts were measured at $\lambda = 673\text{ nm}$ and the concentration of the ZnPTC-OA photosensitizer in the samples were calculated according to a calibration curve.

2.6. Determination of the antibacterial activity of samples of the polyhydroxybutyrate submicron fiber mat loaded with ZnPC-OA

To determine the antimicrobial effects of the fiber mats loaded with the photosensitizer, two different bacterial strains were used. We chose microorganisms that represent different bacterial groups with specific properties for antimicrobial effect of the Zn phthalocyanine derivative testing. Bacteria are ranked into two main classes depending upon their response to the Gram stain which reflects differences in their morphology. Gram-negative and positive bacteria differ in the composition of their cell wall, and respond differently to antimicrobial agents. Bacterial strain *Escherichia coli* CCM 4517 was chosen as a typical representative of Gram-negative bacteria and *Enterococcus faecalis* CCM 4224 as a representative of Gram-positive bacteria. The bacterial strains were purchased from the Czech Collection of Microorganisms, Brno, Czech Republic.

The PHB fiber mats, without (control) and with the ZnPTC-OA photosensitizer, were cut into squares of 50 mm \pm 2 mm. Before the experiments, the squares of the fiber mats were sterilized by gamma-irradiation, using the basic sterilization dose (Microtron MT25, Nuclear Physics Institute, Academy of Sciences of the Czech Republic, Řež).

With the advent of nanotechnology and availability of materials assumed to have antibacterial (antimicrobial) activity, the question arises of how to measure such activity. In the National Reference Laboratory for



Disinfection and Sterilisation (NRL DS), antibacterial (antimicrobial) activity of surfaces of materials is tested in accordance with JIS Z 2801 / ISO 22196 [52]. This internationally recognized test was modified by Textile Testing Institute (Brno, Czech Republic) for determination of antimicrobial activity of photocatalytic surfaces. In short, each of the sterile squares of the fiber mats of 50 ± 2 mm was placed in a sterilized Petri dish making the test surface up. Exactly 0.4 ml of the test inoculum in form of a suspension of the test bacteria in a diluted nutrient broth (500 x diluted for *E. coli*, final bacterial concentration for testing was $4,8 \cdot 10^5$ CFU/ml; 10 x diluted for *E. faecali*, final bacterial concentration for testing was $2,1 \cdot 10^5$ CFU/ml) was pipetted onto the surface of the test material (see Fig. 5). Thereafter the piece with the test inoculum was covered with a square of HDPE foils of (40 ± 2) mm, the foil was pressed so that the test inoculum spreads over the foil while paying attention so that the inoculum does not spill over from the edge of film, and the lid of the Petri dish was placed on (see Fig. 6). The Petri dishes containing the test pieces inoculated with the test inoculum were incubated at a temperature of $(35 \pm 1)^\circ\text{C}$ and a relative humidity of not less than 90 % for (24 ± 1) h in the ATLAS lightbox, equipped by two ATLAS 56001660 tubes, $l = 120$ cm, power 36 W, providing D65 light. D65 roughly corresponds to the colour temperature of the sky on a clear day around noon. Light intensity on the sample surface was (1150 ± 100) lx. Each of the samples was incubated in triplicate under the standard illuminant and unlighted (control).

After 24 h incubation period, the covering film and the test piece were thoroughly washed with 20 ml of tryptone water. Surviving bacteria in the washings were counted to evaluate the antimicrobial activity of the test material. Counts were determined before and after incubation by standard plate count agar cultivation on Petri dishes at 37°C and relative humidity $(95 \pm 2)\%$. Using a formula provided bellow, the log of the difference between the two counts is determined to give a measurement of antimicrobial activity. Difference in between the lowest and the highest result in the triplicate measurements must not exceed 1 log, otherwise the test has to be repeated.

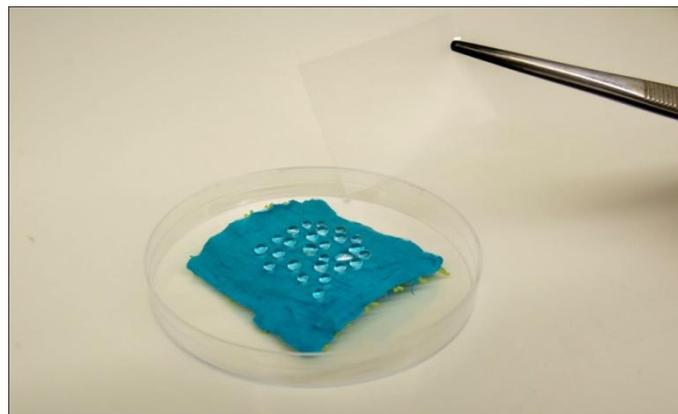


Figure 5: Inoculation of the PHB fiber mat loaded with the ZnPTC-OA photosensitizer by a bacterial suspension

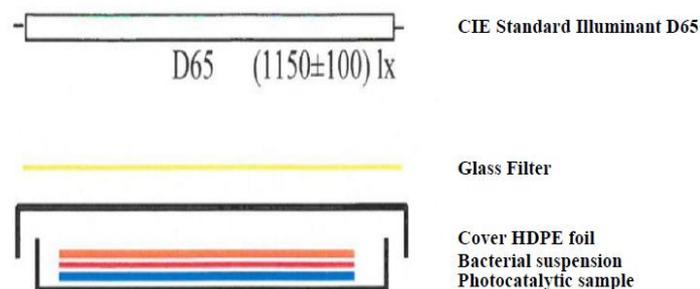


Figure 6: Scheme of the modified JIS Z 2801 assay setup

The antibacterial activity R was calculated according to the formula:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

where,

R is antibacterial activity



U_0 is an arithmetic average of common logarithms of viable bacteria (CFU), determined with 3 control PHB samples immediately after inoculation

U_t is an arithmetic average of common logarithms of viable bacteria (CFU), determined with 3 control PHB samples after 24 h of incubation

A_t is an arithmetic average of common logarithms of viable bacteria (CFU), determined with 3 experimental PHB - ZnPTC-OA samples after 24 h of incubation

3. Results and Discussion

3.1. Results and discussion of singlet oxygen quantification

Results of determination of ZnPTC-OA concentration in different fractions of the ZnPTC-OA enriched PHB fiber mats and singlet oxygen quantification for the pure PHB fiber mat and the ZnPTC-OA enriched PHB fiber mats are summarized in Table 2. The calculated half-time values $\tau_{1/2}$ (s) for the sterilized PHB fiber mat enriched with the ZnPTC-OA photosensitizer (sample 1) and the sterilized (sample 2) and unsterilized (sample 3) cobweb like structures obtained from the tubus of the NANOCENT demonstrator (see Fig. 3) are much shorter than $\tau_{1/2}$ values for DPIBF control and pure PHB fiber mat. The results prove that the ZnPTC-OA enriched PHB fiber samples produce significant amount of highly reactive singlet oxygen (1O_2). Somewhat lower singlet oxygen production rate was determined for the ZnPTC-OA enriched PHB fiber mat (sample 1) produced using the rotating cylindrical collector, in correspondence with lower ZnPTC-OA concentration in the sample. The ZnPTC-OA enriched cobweb like PHB structures (sample 2 and 3) obtained from the tubus of the NANOCENT demonstrator produced the highest amount of singlet oxygen. The sterilization by gamma-irradiation, using the basic sterilization dose, had no negative effect on the singlet oxygen production by the fiber photocatalysts. At this level, gamma-irradiation did not degrade the ZnPTC-OA photosensitizer. No changes have been observed on the PHB polymeric fibers after the irradiation, either. We have not observed any leaching of the ZnPTC-OA derivative from the fibers during our experiments.

Table 2: Singlet oxygen quantification by the DPIBF method in hexan (ar. average \pm SD)

Sample	ZnPTC-OA. [%, w/w]	k (J^{-1})	$\tau_{1/2}$ (s)
DPIBF control	0	0,011 \pm 0,001	1604,5 \pm 159
Pure PHB mat control	0	0,034 \pm 0,001	506,7 \pm 30
Sample 1 (mat from the collector, sterilized)	3,97 \pm 0,12	0,126 \pm 0,01	134,3 \pm 4,0
Sample 2 ("cobweb", sterilized)	4,89 \pm 0,21	0,680 \pm 0,02	28,3 \pm 0,8
Sample 3 ("cobweb", unsterilized)	5,03 \pm 0,23	0,479 \pm 0,01	36,3 \pm 0,7

3.2. Results and discussion of determination of antibacterial activities

Table 3 shows the results of determination of antibacterial activities of the control and ZnPTC-OA loaded fiber mats against Gram-negative *Escherichia coli*. The PHB - ZnPTC-OA submicron fiber mat, obtained with the rotating collector, have been used for the experiment. We have observed very significant antimicrobial effect (3,4 log reduction) of the PHB-ZnPTC-OA submicron fiber mat against *Escherichia coli*, when illuminated by the D65 light source. Even incubation of *Escherichia coli* with the ZnPTC-OA derivative in the dark caused a significant 2,3 log reduction of bacteria counts.

Table 4 shows the results of determination of antibacterial activities of the control and ZnPTC-OA loaded fiber mats against Gram-positive *Enterococcus faecalis*. There was no antibacterial effect in the dark and only moderate effect (1,2 log reduction) was observed under the D65 light source.

The results of the determination of antibacterial activities summarized in tables 3 and 4 are rather surprising. Gram-negative and positive bacteria differ in the composition of their cell wall, and respond differently to antimicrobial agents. Gram-positive bacteria can easily take up molecules such as photosensitizers and can therefore be readily photoinactivated by most photosensitizers used for conventional photodynamic therapy (PDT). This is not the case for Gram negative bacteria which are relatively impermeable to neutral or anionic drugs due to their highly negatively charged surface. Gram-negative bacteria such as *Escherichia coli*, which are typically characterized by an impermeable outer cell membrane that contains endotoxins and blocks antibiotics,



dyes, and detergents, protecting the sensitive inner membrane and cell wall. Gram-positive bacteria possess a porous layer of peptidoglycan and a single lipid bilayer, while Gram-negative bacteria have a double lipid bilayer sandwiching the peptidoglycan layer plus an outer layer of lipopolysaccharide, which results in a low degree of permeability for lipophilic small molecules. The outer membrane of Gram-negative bacteria plays an important role that is related to resistance to many antibiotics that are highly effective against Gram-positive bacteria. This explains the higher prevalence of Gram-negative infections in the modern hospital environment [53]. When the antibacterial efficiency of anionic, cationic and neutral zinc phthalocyanines was tested against Gram-positive and Gram-negative bacteria, the results showed that only the positively charged species, a cationic water soluble pyridinium zinc phthalocyanine was active [11].

The photo-inactivation of Gram-negative bacteria is generally known to be favoured in the presence of the cationic photosensitizers [39]. The localization and subcellular distribution of the phthalocyanines may be a crucial factor in determining their cell killing potential. Therefore, cationic photosensitizers may have a broader application in the photoinactivation of bacterial cells than the anionic or neutral photosensitizers commonly used in photodynamic therapy [11].

In contrast with the results of other research groups discussed above, we have found a very significant antibacterial activity of the neutral ZnPTC-OA derivative, entrapped in PHB submicron fiber matrix, against Gram-negative *Escherichia coli* bacteria under visible light irradiation and even in the dark. On the contrary, the antibacterial effect of the PHB-ZnPTC-OA fiber mat against Gram-positive *Enterococcus faecalis* was only moderate under visible light irradiation and no effect was observed in the dark. We have no unequivocal explanation of the rather surprising results. However, there are several factors, which should be discussed to try to explain the observed phenomena. The first factor is significantly different dilution factor of the nutrition broth used in the antibacterial activity test, which was 10 for Gram-positive *Enterococcus faecalis*, but 500 for Gram-negative *Escherichia coli* bacteria. The dilution factors should be 500 for both the Gram-positive and Gram-negative bacteria according to the original JIS Z 2801/ISO 22196 method [52]. Nevertheless, *Enterococcus faecalis* was not able to survive under light when dilution factor 500 was used. That is the reason why less diluted medium had to be used for the Gram-positive bacteria. More nutrition in the testing suspension had definitely positive effect on survival of bacteria on the sample surface. Moreover, we can't exclude protective effects of the nutrient broth components (beef extracts, peptone, NaCl) against singlet oxygen effects in the case of Gram-positive *Enterococcus faecalis*. For example, other reports show that the incubation of *Escherichia coli* with a cationic phthalocyanine in the dark caused alterations in the outer membrane permeability and increased the uptake of hydrophobic compounds. Adding Mg^{2+} to the cells previous to incubation with a photosensitizer inhibited these alterations in the membrane and also avoided the photodynamic inactivation (PDI) of the bacteria [54]. Further, natural antioxidants can have protective effects against reactive oxygen particles.

The second factor, which could influence the antibacterial activity of the tested photocatalytic material is the selected polymeric matrix, used for the photosensitizer entrapment. However, there is no systematic review or discussion on this subject until now.

PDT or PDI are new promising strategies to eradicate pathogenic microorganisms such as Gram-positive and Gram-negative bacteria, yeasts and fungi. The search for new approaches that can kill bacteria but do not induce the appearance of undesired drug-resistant strains suggests that PDT may have advantages over traditional antibiotic therapy. Antimicrobial PDT is particularly good for dental and dermatological applications, involving the light irradiation of a tissue containing microorganisms that were previously exposed to a photosensitizing dye.

Table 3: Results of determination of antibacterial activities of the control and ZnPTC-OA loaded fiber mats against *Escherichia coli*.

Sample	Time 0 h	Time 24 h	Antimicrobial activity R
PHB control (lighted)	$U_0 = 4,0$	$U_{t-LIGHTED} = 4,7$	-
PHB control (unlighted)	$U_0 = 4,0$	$U_{t-UNLIGHTED} = 5,6$	-
PHB + ZnPTC-OA (lighted)	-	$A_{t-LIGHTED} = 1,3$	3,4
PHB + ZnPTC-OA (unlighted)	-	$A_{t-UNLIGHTED} = 3,3$	2,3



Table 4: Results of determination of antibacterial activities of the control and ZnPTC-OA loaded fiber mats against *Enterococcus faecalis*

Sample	Time 0 h	Time 24 h	Antimicrobial activity R
PHB control (lighted)	$U_0 = 3,9$	$U_{t-LIGHTED} = 5,9$	-
PHB control (unlighted)	$U_0 = 3,9$	$U_{t-UNLIGHTED} = 5,9$	-
PHB + ZnPTC-OA (lighted)	-	$A_{t-LIGHTED} = 4,7$	1,2
PHB+ ZnPTC-OA (unlighted)	-	$A_{t-UNLIGHTED} = 6,0$	0



Figure 7: The PHB submicron fiber mats, loaded with the Zn phthalocyanine derivative, prepared by the centrifugal spinning technology using the rotating fiber collector

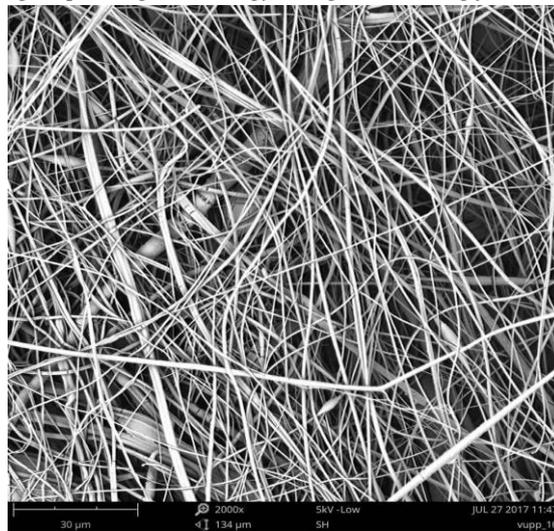


Figure 8: SEM image of microstructure of the PHB submicron fiber mat loaded with the Zn phthalocyanine derivative

4. Conclusions

The results proved a significant production of singlet oxygen by the PHB-ZnPTC-OA submicron fiber mat prepared by the centrifugal spinning technology. This alternative approach allows to fabricate nanofibers or microfibers from solutions at high speed and low cost. In contrast with the results of other research groups published before, we have found a very significant antibacterial activity of the neutral zinc Ptc derivative, entrapped in PHB submicron fiber matrix, against Gram-negative *Escherichia coli* bacteria under visible light irradiation and even in the dark.



In view of the prediction of the “end of the antibiotic era”, antimicrobial PDT is starting to be considered as a promising alternative approach to resistant infections and has the further advantage of not leading to the selection of resistant strains. Gram-negative bacteria are responsible for many life-threatening infections in humans and they are often innately resistant to the most commonly used antibiotics, making the search for new antibacterial drugs and alternative therapies, such as PDT, very important. The PHB-ZnPTC-OA submicron fiber mat with significant activity against Gram-negative *Escherichia coli* bacteria has interesting properties as a candidate for PDT, PDI, environmental or other photocatalytic applications. Because of biodegradability and resorbability of the PHB biomaterials, it is especially suitable for medical applications.

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