Optical biosensors with porphyrins immobilized into polysilsesquioxane matrices

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Introduction

Porphyrins are macrocyclics with important optical properties, such as the great absorptions of visible light (the extinction coefficient ~ 10^5 $M^{-1}cm^{-1}$), the high values of excitation lifetimes up to 10 ms for phosphorescence and more than 10 ns for fluorescence and high quantum yields of singlet oxygen. These optical properties might be applied in constructions of optical chemical sensors and materials with antimicrobial and photocatalytic effects. These applications need a fixation of porphyrins in a transparent, mechanically and chemically stable carrier that is porous enough to ensure transport of oxygen or other analytes. Silica matrices prepared by sol-gel process are good candidates but these carriers suffer from low reproducibility of porosities and specific surfaces. Polysilsesquioxanes, which can be also prepared by the sol-gel proccess have better defined porosity and mechanical properties (Oviatt et al., 1993).
In this work we immobilized hydrophilic porfyrin TMPyP and hydrophobic porphyrin TPP into polysilsesquioxane matrices. We compared optical properties of the newly-synthesized polysilsesquioxane layers, such as fluorescence, absorbance and singlet oxygen generation, with commonly used silica matrix containing TMPyP and poly(dimethylsiloxane) with entrapped TPP. Optical properties of new porphyrin films were correlated with their BET surface area, pore diameter and film thickness.

Material and methods

Chemicals, strains
Chemicals: 5,10,15,20-tetrafenylporphyrin (TPP); 5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphyrin tetra(toluen-4-sulphonate) (TMPyP); tetramethoxysilane (TMOS) were purchased from Fluka. Silicon elastomer Sylgard 184 (PDMS) was from Dow Corning. Ethanol (96 %); toluene p.a., NaOH and HCl were obtained from P-LAB.
Microorganisms: GMO *Escherichia coli* BL21 strain, producing red fluorescent protein dsRed, ampicillin resistant was obtained from DBM of ICT Prague, CZ.

Monomers preparation
Monomers of 1,2-bis(triethoxysilyl)ethane (BTE_M), 1,6-bis(triethoxysilyl)hexane (BTH_M) and 1,8-bis(triethoxysilyl)octane (BTO_M) were synthesized by method published in Oviatt et al. (1993) and their purity was controlled with GC.

Prepolymerization
Polysilsesquioxane: Monomers were mixed with ethanol and NaOH solution (1M) in ratios shown in Tab. 1 at 25 ^oC.
TMOS: TMOS was mixed with deionized water and HCl in TMOS:H_2O:HCl mol ratio = $1:5:10^{-2}$ to form a clear solution and left to prepolymerize at 4^oC for 24 h.

PDMS: PDMS and cross-linker were mixed in 10:1 ratio (w/w) in toluene (80%). This solution was heated at 60 ^{o}C for 30 min (van Laar, 2001).

Porphyrins immobilization

Prepolymers were mixed with TPP solution in toluene (10^{-4}M) and TMPyP solution in deionized water (10^{-4}M) in 9:1 (v/v) ratio in combinations shown in Tab. 2. $TMOS_{PP}$+TMPyP mixture was mixed with NaOH solution (0,05M) in 1:0.35 (v/v) ratio. All layers were prepared by pouring of prepolymer-porphyrin mixture (1 ml) onto a glass Petri dish (ø 3 cm) and kept in dark at 4 ^{o}C. Each sort of layer was prepared in three parallels. After gellification (< 2 min), the TMOS layers were poured over by deionized water. For antimicrobial test polysilsesquioxane films, polymerizing at 25 ^{o}C for 16 h, were poured over by water. PDMS layers, polymerizing at 25 ^{o}C for 16 h, were dried in vacuum for 4 h.

monomer (purity %)	BTE_M (90%)	BTH_M (95%)	BTO_M (95%)
monomer [g]	0,393	0,432	0,462
ethanol (96%) [ml]	5,2	5,2	5,2
1M NaOH [drop]	4	10	10
time of mixing [min]	60	45	30

Tab. 1: Ratios of reactants and time of mixing used for polysilsesquioxane pre-polymerization.

porfyrin / prepolymer	TMPyP	TPP
BTE_{PP}	+	+
BTH_{PP}		+
BTO_{PP}		+
$TMOS_{PP}$	+	
$PDMS_{PP}$		+

Tab. 2. Prepolymer-porfyrin combinations for layers preparation.

Measurement of optical properties

Absorbance: Absorbance spectra of prepared layers were measured with HP 8452 A. Light scatter was corrected using Scatter correction function in Advance mode of UV/Vis ChemStation software (Agilent Technol. 95-00) at 350-400 nm and 450-800 nm. Absorbance intensity of Soret peak maximum of TPP immobilized into PDMS, BTE, BTH and BTO at 414, 414, 415 and 416 nm, resp. and TMPyP entrapped into TMOS and BTE matrices at 430 nm were statistically evaluated.

Fluorescencet: 3D-fluorescence spectra of prepared layers were collecting using Hitachi F-4500. Fluorescence intensity of each sort of immobilized TPP at 410nm/650nm and TMPyP at 430nm/650nm (EX/EM) were statistically evaluated.

Thickness of layers

TMOS-TMPyP layers and PDMS-TPP layers were measured using micrometer SOMET (CZ). Polysilsesquioxane layers were measured with Olympus BX51 microscope.

BET and porosity measurement of xerogels

BTE, BTH, BTO, TMOS and PDMS polymer without porphyrins were dried at 40 ^{o}C for 3 h. Further xerogels were dried in 10^{-7}Pa vacuum at 40 ^{o}C for 24 h. BET surface area and average pore diameter (4V/A by BET) were measured using ASAP 2010M (Micromeritics, USA).

Antimicrobial activity test

E. coli BL21 was cultivated in LB broth (1 % w/v) with ampicillin (100 mg/l) at 37 ^{o}C for 16 h. Inoculum was diluted to the concentration of 10^4 CFU/ml into LB agar (1 % w/v) with ampicillin (100 mg/l). Inoculated LB agar (1 ml) was poured over a porphyrin-film at the bottom of a Petri dish. The Petri dishes were illiminated by 300W halogen lamp from distance of 70 cm for 0; 1.5 and 3 h, resp. After illumination, the inoculated Petri dishes were incubated in dark at 37 ^{o}C for 48 h.

Results and discussion

Polymers without porphyrins were characterised by porosity and BET surface area measurement (Fig. 1). PDMS had no porosity and negligible surface area in contrast to TMOS with high surface are due to large content of micropores (average pore diameter of 20 A). Polysilsesquioxanes are mainly mesoporous, although BTE has a quantum of micropores.

We entrapped hydrophobic porphyrin TPP into three polysilsesquioxanes BTE, BTH, BTO and PDMS. In TMOS, TPP was insoluble. Hydrophilic porphyrin TMPyP was entrapped only into BTE and TMOS because TMPyP was insoluble in matrices which contain a longer aliphatic chain and thus are more hydrophobic and also less fragile.

Immobilization of TMPyP into hydrophilic matrices leads to bathochromic shift of Soret peak. In films maximum of Soret peak was λ = 430 nm and in water solution λ = 422 nm. TMPyP leaked from BET films (~ 10%), while in TMOS was firmly fixed. Strong interactions of cationic TMPyP and anionic TMOS matrix are manifested in significant change of UV/VIS spectra. Maximum fluorescence of these layers was EX/EM 430nm/650nm.

TPP immobilized into hydrophobic matrices showed hypsochromic shifts, +2 nm, against the toluene solution of TPP. These spectral shifts occur probably due to hydrophobicity of matrices. Fluorescent maxima were found at 410nm/650nm (EX/EM) (Fig.2.).

Higher film porosity that resulted in higher film fluorescence (Fig. 3) led also to higher antimicrobial activity that was demonstrated by decreasing of number of colonies on illuminated agar with porphyrin film. PDMS with TPP has no antimicrobial effect, whereas BTH with TPP has the highest one.

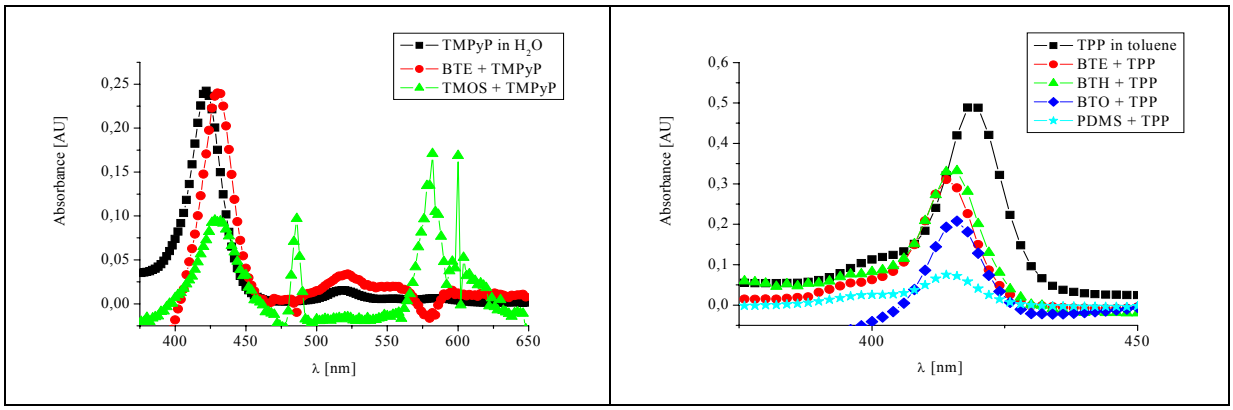

Fig. 2. Spectra of porphyrin-polymer layers and TMPyP solution in deionized water and toluene solution of TPP.

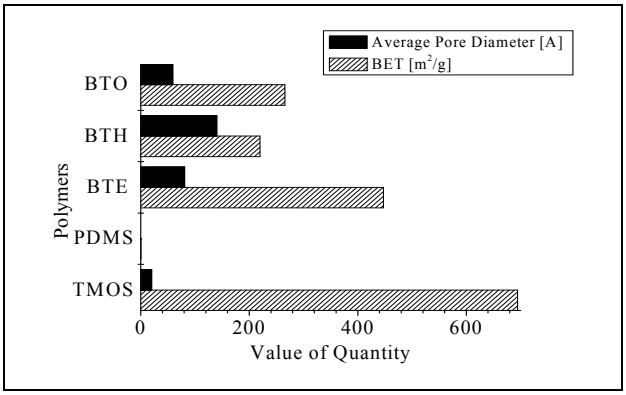

Fig. 1. Polymers porosity characterisation.

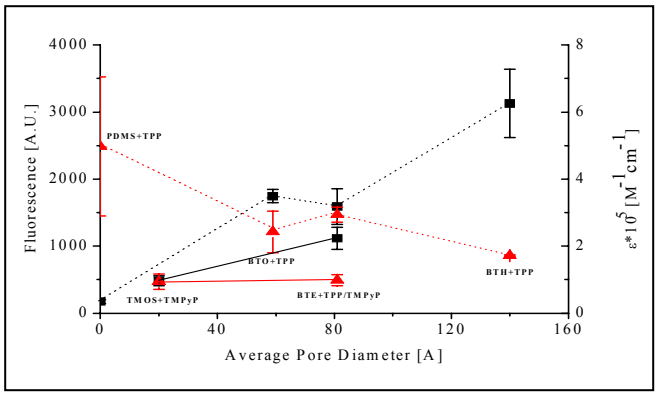

Fig. 3. Intensity of fluorescence and extinction coefficient as a function of pore diameter for polymer-porphyrin layers (■ fluorescence, ▲ ε, __ hydrophilic layers, ... hydrophobic layers).

Conclusions

The newly-synthesized matrices BTE, BTH and BTO with immobilized hydrophilic porfyrin TMPyP and hydrophobic porphyrin TPP performed the higher fluorescence and antimicrobial activity based on generation of the singlet oxygen, as compared with TMOS-TMPyP matrix and PDMS-TPP matrix. These effects are due to mesoporous structure of polysilsesquioxanes. In addition, the resilience of polysilsesquioxanes BTE, BTH and BTO makes them more favourable for sensor design.

References

Oviatt H. W., Jr. et al. (1993) *Alkylene-Bridged Silsesquioxane Sol-Gel Synthesis and Xerogel Characterization. Molecular Requirements for Porosity*. Chem. Mater. 5: 943-950.

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Acknowledgements

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The materials were characterised by their texture and optical properties (fluorescence and absorbance) and by light-induced antimicrobial activity against E. coli BL21(DE3) (pET16bDsRed) strain.Â @article{Rychtrikov2012PhotodynamicEO, title={Photodynamic efficiency of porphyrins encapsulated in polysilsesquioxanes}, author={Renata Rycht{\'a}rikov{\'a} and Stanislav {\vS}abata and JiÅ™{\'i} Hetflej{\vs} and Gabriela Kuncov{\'a}}, journal={Chemical Papers}, year={2012}, volume={66}, pages={269-277} }. A biosensor is an analytical device containing an immobilized biological material (enzyme, antibody, nucleic acid, hormone, organelle or whole cell) which can specifically interact with an analyte and produce physical, chemical or electrical signals that can be measured. An analyte is a compound (e.g. glucose, urea, drug, pesticide) whose concentration has to be measured. Biosensors basically involve the quantitative analysis of various substances by converting their biological actions into measurable signals. A great majority of biosensors have immobilized enzymes. The performance of the bios...