

Fluorescence properties of sol–gel materials doped with photosensitizers

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Sol–gel materials may find plenty of applications, *e.g.*, as carriers for various substances, which can be exploited for sensing purposes or as drug releasing carriers. In this work, the fluorescence properties of two photosensitizers immobilized in silica based sol–gel materials were examined. The sol–gel materials were produced from silica precursor TEOS and 96% ethyl alcohol as solvent. Materials with various ratios R (solvent to precursor molar ratio) 5, 15, 20, 32, 40, and 50, were prepared. Photolon and protoporphyrine PPIX were used as dopants in concentrations 0.001%, 0.005%, 0.01% and 0.05%. The excitation wavelength from a blue laser was 415 nm. The highest luminescence was observed for the material prepared with $R = 20$, whereas for $R = 5$ it was the lowest one. The fluorescence quenching for highest photosensitizer concentration was observed, as well.

Keywords: fluorescence, sol–gel material, photosensitizers.

1. Introduction

Macrocyclic molecules play key roles in basic processes in living organisms. Porphyrins exhibit a wide range of important optical properties. In these systems, the position of the most intense absorption band depends on the peripheral substituents of the macrocycle. Generally, sol–gel method allows the successful entrapping of porphyrins into inorganic networks. The materials obtained are robust and transparent monolithic gels, but in the majority of cases the red fluorescence of the porphyrins disappears with ageing [1]. The type and spatial disposition of the substituents in the porphyrin macrocycles were evaluated with the emphasis on the conservation of red fluorescence of porphyrins entrapped or covalently bonded to the inorganic sol–gel SiO₂ matrix [2, 3].

Porphyrins are known to be sensitive to metal ions, because both, absorption and fluorescence change is observed under complexation of ions such as mercury, lead, cadmium [4]. Porphyrins can serve as probe molecules of various other compounds

[5, 6]. The study of the dimer formation of organic dopants in sol–gel materials has received relatively little attention, despite the fact that monomer–dimer equilibria determine the optical and photochemical behavior of the organic dyes [7]. Moreover, the porphyrins are versatile molecules whose physicochemical properties can be adjusted by modifications of the electronic distribution on the aromatic ring. Fluorescent properties of the europium porphyrin in silica sol–gel matrices make this complex useful for some special applications as sensing of molecular oxygen or biomolecules [8]. We have also demonstrated that porphyrins entrapped in sol–gel matrices preserve their chemical activity showing clear response to changes in the environment, what can be seen in the fluorescence spectra [9]. The chemical activity was also checked in *in vitro* study on antibacterial photodynamic action with sol–gel applicators [10]. It is known that concentration of photoactive agents in the porous matrix influences the fluorescence intensity, however, when designing sensors or applicators, the phenomenon of concentration quenching should be taken into account [11]. In this work we study the fluorescence of doped sol–gel materials depending on material composition (R ratio) and dye concentration.

2. Material

The sol–gel materials were prepared from silicate precursor tetraethylorthosilicate TEOS (Fluka), ethyl alcohol 96% (Polish Chemicals) as solvent, and detergent Triton X-100 (Aldrich). The corresponding amount of solvent was used in order to obtain the required ratios R ($R = 5, 10, 15, 20, 32, 40$ and 50), denoting the number of ethanol moles to the number of TEOS moles. Hydrochloric acid 37% (Polish Chemicals) was added as a catalyst to ensure acid hydrolysis ($\text{pH} \approx 2$). The mixture was stirred at room temperature for 4 hours using a magnetic stirrer (speed 400 rpm).

Photolon (18-carboxy-20-(carboxymethyl)-8-ethenyl-13-ethyl-2,3-dihydro-3,7,12,17-tetramethyl-21H, 23H-porphin-2-propionic acid) (Belmedpreparaty in cooperation with Haemato Ltd.) and protoporphyrine IX dimethylester (dimethyl-8,13-divinyl-3,7,12,17-tetramethyl-21H, 23H-porphine-2,18-dipropionate) (Fluka) were used as photosensitive dyes. Stock solutions 0.5% of photosensitive dye were prepared by dissolving the photosensitizer in ethyl alcohol. The adequate amounts of solutions were added to 1 ml of the fresh prepared sol in order to get the final concentrations of the dyes: 0.001%, 0.005%, 0.01% and 0.05%.

3. Method

The blue laser from TOP–GaN (Poland) emitting 415 nm was used as an excitation source, what fits the absorption band of our photosensitizers [12]. Fluorescence spectra were obtained by means of the spectrophotometer Ocean Optics SD 2000. Spectra were recorded in the wavelengths range from 350 nm to 900 nm. Fluorescence spectra were measured for the freshly prepared samples, 24 hours after the end of stirring process. The samples were stored in darkness and at room temperature. Sols

were placed into the plastic cuvettes (optical length 0.5 cm, Sigma) and illuminated from above. Emitted light was captured from side, at the angle 90° to laser beam and guided by the fiberoptic cable to the detector. For each sample, the spectra were recorded four times from every side of the cuvette (the cuvette was rotated each 90°). The spectra represent the mean values of the recorded fluorescence intensity normalized to the exciting light intensity.

4. Results

4.1. Sol-gel materials doped with protoporphyrine IX

The analysis of the recorded spectra has revealed the fact that the fluorescence intensity strongly depends on the molar ratio R . The strongest fluorescence was recorded for $R = 20$. Fluorescence spectra of the sol-gel samples prepared with

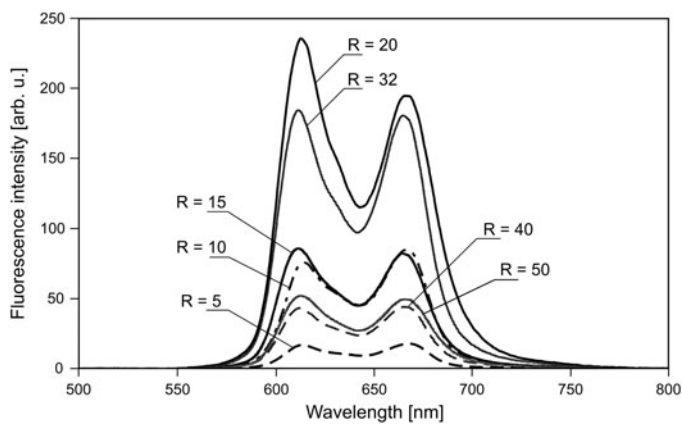


Fig. 1. Fluorescence spectra of sol-gel materials doped with PPIX, concentration 0.05%.

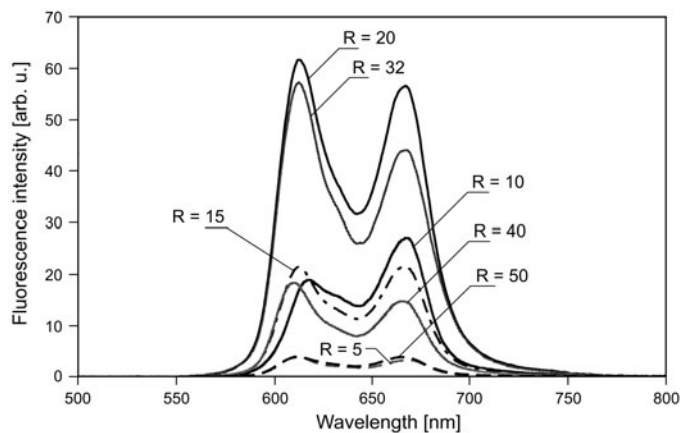


Fig. 2. Fluorescence spectra of sol-gel materials doped with PPIX of concentration 0.001%.

various R ratios doped with PPIX (concentration 0.05%) are depicted in Fig. 1. For all measured samples emission maxima in the range 612–616 nm and 666–669 nm were detected.

For comparison, in Fig. 2 the emission spectra of PPIX in the lowest concentration (0.001%) are shown. At 612 nm a strong fluorescence maximum is detected. Additional maximum was found at 668 nm. Again, the highest intensity was observed for material prepared with ratio $R = 20$. The similar results are observed for other concentrations of the photosensitive dyes.

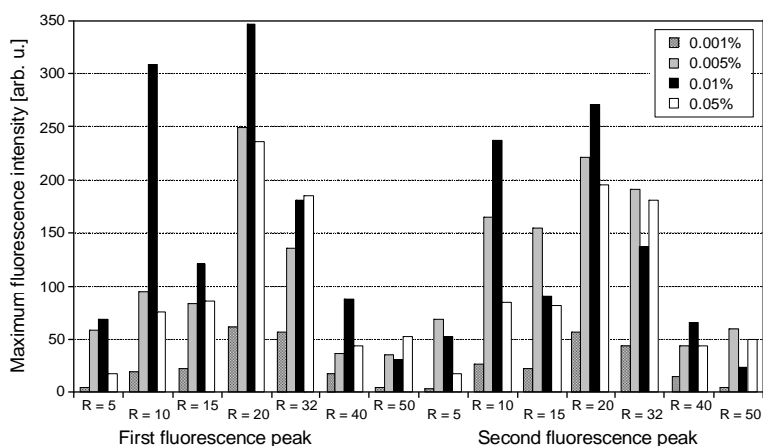


Fig. 3. Maximal fluorescence intensity of sol-gel materials prepared with various R doped with various concentrations of PPIX.

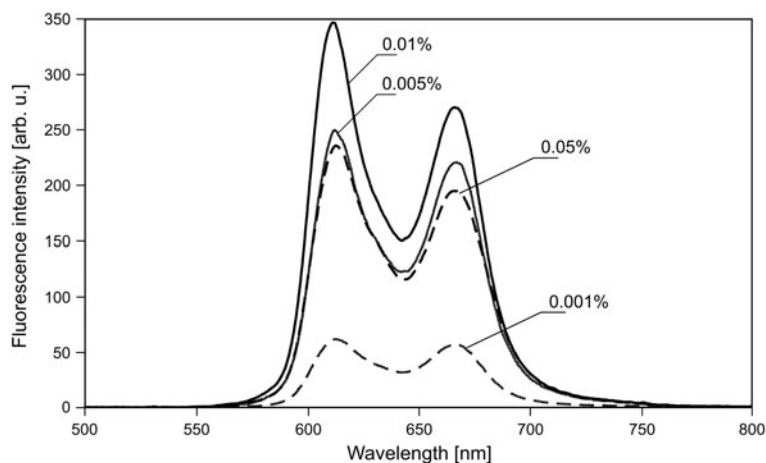


Fig. 4. Fluorescence spectra of sol-gel materials prepared with $R = 20$, doped with PPIX, various concentrations.

The intensities of fluorescence maxima are depicted in the diagram in Fig. 3. This diagram clearly demonstrates that for each concentration the highest emission intensity occurs for the material prepared with $R = 20$. For each R the concentration depended intensity quenching is observed, as well. Moreover, the R depended quenching is also visible (the intensity decreases for R higher than 20).

Diffused maxima presented in Figs. 1 and 2 resulted from specific interaction between solvent (in this case ethyl alcohol) and dimerized form of protoporphyrine. Moreover, low intensity of fluorescence denotes that typical dimer form dominates, however, other forms of the photoactive agent are present, as well.

The concentration depended quenching is demonstrated in Fig. 4, presenting the fluorescence spectra of sol-gel materials prepared with ratio $R = 20$, doped with PPIX in various concentrations. One can see that the fluorescence depends on the concentration of photosensitizer. The lowest one was recorded for 0.001%, whereas, the highest was for 0.01% PPIX concentration. This result clearly shows that for the highest concentration the fluorescence quenching occurs.

4.2. Sol-gel materials doped with photolon

The same analysis was performed for photolon doped sols. Fluorescence spectra of various sols doped with photolon in the highest concentration 0.05% are demonstrated in Fig. 5. Figure 6 presents the results for the lowest concentration 0.001%. Emission maxima at 654–668 nm and a shoulder at 705–706 nm were detected. Photolon in ethyl alcohol is partially dimerized, so one can observe one main peak and shoulder. The fluorescence maximum is shifted in the direction of longest wavelengths for photolon in concentration 0.05%.

Analyzing Fig. 7, one can see that the intensity increases starting from $R = 5$ up to $R = 20$, and then decreases for R higher than 20. The concentration fluorescence

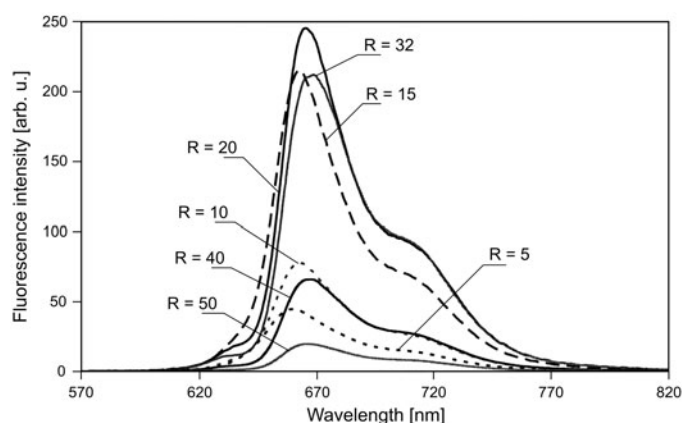


Fig. 5. Fluorescence spectra of sol-gel materials, doped with photolon, concentration 0.05%.

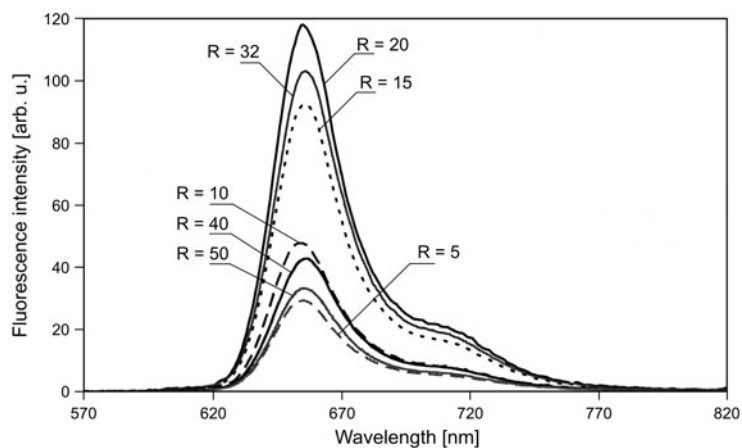


Fig. 6. Fluorescence spectra of sol-gel materials, photolon in concentration 0.001%.

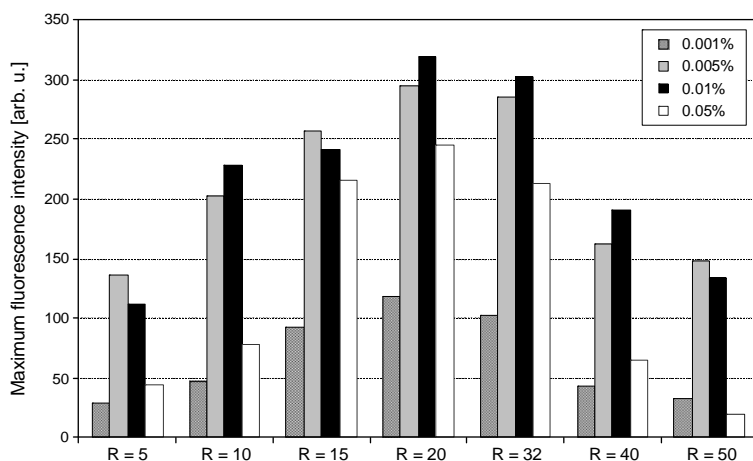


Fig. 7. Maximal fluorescence intensity of sol-gel materials prepared with various R , doped with various concentrations of photolon.

quenching of materials prepared with $R = 20$ is visible, what is also demonstrated in Fig. 8.

In doped sol-gel material with $R = 20$ the highest emission intensity is observed at 665 nm for photolon in concentration 0.01%.

5. Conclusions

This study demonstrates that the photosensitive agents entrapped in sol-gel material preserve their chemical activity. The fluorescence intensity of protoporphyrine IX and photolon is influenced by the molar ratio R of sol-gel materials. This means that

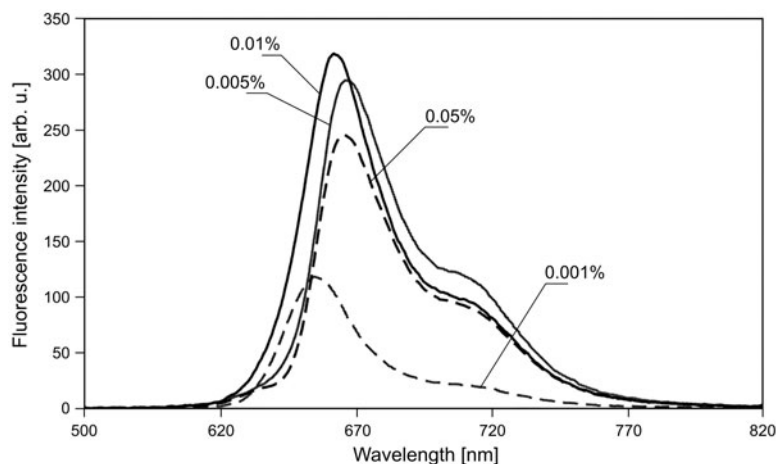


Fig. 8. Fluorescence spectra of sol-gel materials prepared with $R = 20$, doped with PPIX, various concentrations.

spectroscopic parameters of sol-gel materials doped by photosensitizers depend on alcohol content in sol. The materials prepared with ratio $R = 20$ show the highest emission intensity. The fluorescence intensity depends on the dyes concentration, too. The concentration depended quenching was observed as well. These results should be taken into consideration when designing the sol-gel fluorescence based sensors or applicators for photodynamic therapy.

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*Received September 18, 2007
in revised form February 9, 2008*