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# LuAG:$Pr^{3+}$-porphyrin based nanohybrid system for singlet oxygen production: Toward the next generation of PDTX drugs

Kseniya Popovich[a], Kateřina Tomanová[a,b], Václav Čuba[a,*], Lenka Procházková[a,b], Iveta Terezie Pelikánová[a], Ivo Jakubec[c], Eva Mihóková[b], Martin Nikl[b]

[a] Department of Nuclear Chemistry, Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague, Břehová 7, Prague 115 19, Czech Republic
[b] Department of Optical Materials, Institute of Physics of the Czech Academy of Sciences, Cukrovarnická 10, Prague 162 53, Czech Republic
[c] Institute of Inorganic Chemistry of the Czech Academy of Sciences, Husinec-Řež 1001, Řež 250 68, Czech Republic

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ABSTRACT

A highly prospective drug for the X-ray induced photodynamic therapy (PDTX), LuAG:$Pr^{3+}$@$SiO_2$-PpIX nanocomposite, was successfully prepared by a three step process: photo-induced precipitation of the $Lu_3Al_5O_{12}$:$Pr^{3+}$ (LuAG:$Pr^{3+}$) core, sol–gel technique for amorphous silica coating, and a biofunctionalization by attaching the protoporphyrin IX (PpIX) molecules. The synthesis procedure provides three-layer nanocomposite with uniform shells covering an intensely luminescent core. Room temperature radioluminescence (RT RL) spectra as well as photoluminescence (RT PL) steady-state and time resolved spectra of the material confirm the non-radiative energy transfer from the core $Pr^{3+}$ ions to the PpIX outer layer. First, excitation of $Pr^{3+}$ ions results in the red luminescence of PpIX. Second, the decay measurements exhibit clear evidence of mentioned non-radiative energy transfer (ET). The singlet oxygen generation in the system was demonstrated by the 3′-(*p*-aminophenyl) fluorescein (APF) chemical probe sensitive to the singlet oxygen presence. The RT PL spectra of an X-ray irradiated material with the APF probe manifest the formation of singlet oxygen due to which enhanced luminescence around 530 nm is observed. Quenching studies, using $NaN_3$ as an $^1O_2$ inhibitor, also confirm the presence of $^1O_2$ in the system and rule out the parasitic reaction with OH radicals. To summarize, presented features of LuAG:$Pr^{3+}$@$SiO_2$-PpIX nanocomposite indicate its considerable potential for PDTX application.

## 1. Introduction

In recent decades the search for new luminescence, phosphor and scintillation materials intensified due to increasing number of their applications. A prominent field of interest focuses on medical and biomedical applications, such as imaging systems for medical diagnosis [1–9]. Particularly intriguing challenge is represented by an effort to push the limit of coincidence resolution time in the time-of-flight positron emission tomography [10–12] toward 10 ps [13]. Achieving that goal would significantly improve an effective sensitivity, provide better image quality, reduce the scan times, dose and costs and would eliminate the need for image reconstruction. On the other hand the task cannot be fulfilled with conventional scintillators and new approaches providing ultrafast scintillating materials need to be investigated [14–16].

Alternative research direction of scintillator applications in medicine focuses on their exploitation in medical therapy. X-ray induced photodynamic therapy (PDTX) uses tumor-destroying agents based on scintillating nanoparticles (NP) conjugated with photosensitizer (PS) molecules. The agent accumulates preferentially in the target cells; subsequently, the external X-ray irradiation excites the scintillating NP, emitting secondary radiation, which activates the PS molecules [17–21]. Their deexcitation via non-radiative energy transfer (ET) leads to the production of the reactive oxygen species, where the singlet oxygen is believed to be the most cytotoxic [22,23]. To observe a cytotoxic effect at therapeutic radiation doses, the light yield of scintillating NP, the efficiency of energy transfer to the photosensitizer and the cellular uptake of the nanoparticles, all need to be fairly well optimized [24].

The principal advantage of PDTX with respect to conventional PDT [25,26] is that deeply residing tumors can also be treated [27,28] while keeping surrounding tissues at the low level of irradiation. PDTX combines advantages of both photodynamic therapy and radiotherapy to achieve the most efficient treatment. The preparation of the X-ray induced photodynamic therapy drug includes four key steps:

* Corresponding author.
*E-mail address:* vaclav.cuba@fjfi.cvut.cz (V. Čuba).

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1. Synthesis of nanoparticles with suitable chemical, morphological and luminescence characteristics;
2. Nanoparticle surface modification by various mechanisms. The most commonly used techniques are core–shell (e.g. $EuF_3$@$GdF_3$) or surface covering with amorphous silica layer [25];
3. Conjugation of the nanoparticle with photosensitizer molecules. The process is also referred to as functionalization;
4. Improvement of the drug action either by enhanced permeability and retention (EPR) effect or by conjugating a molecule that can target some specific antigens of tumor cells [17].

PDTX treatment requires application of composite materials with the core made of chemically stable, non-toxic nanoparticles with low reactivity and high $Z_{eff}$ and average size in the range of a few to hundreds of nm [29, 30] to promote EPR effect. From this point of view, synthetic garnets such as lutetium aluminum garnet ($Lu_3Al_5O_{12}$, LuAG) seem to be highly prospective. Structurally, the garnets correspond to the naturally occurring $R_3^{II}M_2^{III}(SiO_4)_3$ silicates with a cubic crystal structure and $Ia\bar{3}d$ space group. Nanocrystalline LuAG has extremely high chemical stability, thermal stability up to 2000 °C and a cubic structure, preventing mechanical damage to the tissue. It has high density (6.73 g/cm$^3$), high effective atomic number ($Z_{eff}$ = 58.9), radiation stability and high luminescence intensity [31,32], reaching maximum at crystallite size about 30–50 nm. Doping with $Pr^{3+}$ ions ensures radioluminescence spectrum overlapping with absorption bands of photosensitizing protoporpyrin IX (PpIX) [33,34]. PpIX is naturally present in living cells in small amounts as a precursor of heme.

The synthesis and singlet oxygen production has already been demonstrated on hybrid systems containing e. g. ZnO [27], $Y_2O_3$ [35], $Tb_2O_3$ [18] and $CeF_3$:$Tb^{3+}$ [36] as a scintillating core. The critical step of nanocomposite preparation always resides in binding a layer of photosensitizer to the surface of scintillating nanoparticle to form a homogeneous shell. However, this is increasingly more difficult if the core particle size exceeds 1–5 nm and becomes yet more challenging if the core exhibits extremely low reactivity. To solve the problem, one may adopt an alternative approach. First, the scintillating core is coated with a uniform shell of amorphous silica. When completed, one proceeds by binding a photosensitizer. As a result, three-layer nanocomposite is formed.

In this work, we present a concept of preparation of LuAG:$Pr^{3+}$@$SiO_2$-PpIX nanocomposites for PDTX application. Using the photo-induced method [31] we synthesize intensely luminescent LuAG:$Pr^{3+}$ nanoparticles with an average size of about 30 nm. Subsequently, we perform the surface coating procedure with $SiO_2$ amorphous layer via sol–gel process. Finally, LuAG:$Pr^{3+}$ nanoparticles are conjugated with a photosensitizer molecule (PpIX). By methods of optical spectroscopy we demonstrate the presence of energy transfer between the nanoparticle core and outer photosensitizing layer. We also address the potential of presented nanocomposite in PDTX application by testing its ability of the singlet oxygen production.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Core Synthesis: LuAG:$Pr^{3+}$

LuAG:$Pr^{3+}$(1%) was prepared using photo-induced method described by Bárta et al. [31]. An initial solution containing $3{\cdot}10^{-3}$ mol/L of lutetium nitrate hydrate (99.999%, Sigma-Aldrich), $5{\cdot}10^{-3}$ mol/L of aluminum nitrate nonahydrate (99.997%, Sigma-Aldrich), 0.1 mol/L of ammonium formate (≥99.995%, Sigma-Aldrich) and $3{\cdot}10^{-5}$ mol/L of $Pr(NO_3)_3$ was prepared in deionized water. For $Pr^{3+}$ doping, $Pr(NO_3)_3$ stock solution was prepared by dissolving of $PrO_{2-x}$ (≥99.999%, Koch-Light Laboratories) in concentrated nitric acid. The irradiation was performed in a photochemical reactor, which was continuously stirred and cooled by water. As a light source, UVH 1016-6 medium-pressure mercury lamp (UV Technik Meyer GmbH, Germany) was used. The power input of the lamp was set to 360 W and the reaction mixture was irradiated for 3.5 h.

After irradiation the obtained gelatinous suspension was filtered from the solution by microfiltration using 0.45 µm HAWP membrane filter (Millipore Ltd.). The precursor was dried at 40 °C in air for 24 h. The precursor was suspended in ethanol (96%, p.a., PENTA) in an ultrasonic bath for 3 h and was left to dry at 40 °C in air for 24 h. Finally, the nanocrystalline $Pr^{3+}$-doped LuAG was obtained by calcination at 1000 °C for 2 h (10 °C/min) in a 0415 VAK vacuum furnace (Clasic).

#### 2.1.2. Encapsulation in Amorphous Silica Layer – Surface Modification Process: LuAG:$Pr^{3+}$@$SiO_2$

The prepared LuAG:$Pr^{3+}$ nanopowder was coated by a silica layer using a modified sol–gel process described by Liu et al. [37]. 175 mg of the nanopowder was heated in a vacuum furnace to 120 °C for 1 h. After cooling down to the room temperature in a desiccator, the dry nanopowder was suspended in 40 mL of absolute ethanol (≥99.8%, p.a., PENTA) in an ultrasonic bath for 1 h.

The suspension was placed on a magnetic stirrer and 20 µL of tetraethoxysilane (TEOS; ≥99%, Sigma-Aldrich) was added. After that, 6 mL of ammonium hydroxide solution (25–29%, p.a., PENTA) was slowly added to the mixture in a dropwise manner. The solution was left overnight under stirring to ensure the maximum hydrolysis of TEOS, which is necessary to form a silica layer. The silica-coated nanoparticles were rinsed 3× in water and subsequently suspended in ethanol in an ultrasonic bath for 1 h. The suspension of nanoparticles was left to dry at 40 °C in air for 24 h.

#### 2.1.3. Outer Layer of the Nanocomposite – Functionalization Process: LuAG:$Pr^{3+}$@$SiO_2$-PpIX

The prepared silica-coated LuAG:$Pr^{3+}$ nanoparticles were conjugated with PpIX using a modified biofunctionalization procedure reported by Nowostawska et al. [38]. In this method, the functional amino groups are first attached to PpIX and then the $(NH_2)$n-PpIX are conjugated with the surface of silica-modified nanoparticles. 100 mg of PpIX (≥95.0%, Sigma-Aldrich) were dissolved in 20 mL of THF (p.a., PENTA). Subsequently, 132 µL of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide (≥97.0%, Sigma-Aldrich) were added, and the solution was stirred for 3 h at 0 °C in inert atmosphere. Then, 83 µL of (3-aminopropyl)triethoxysilane (≥97.0%, Sigma-Aldrich) in 10 mL of THF were added to the mixture at 0 °C, and the reaction proceeded for 8 h at room temperature.

100 mg of LuAG:$Pr^{3+}$@$SiO_2$ nanopowder and 300 µL of triethylamine (≥99%, Sigma-Aldrich) were added to the prepared solution and a suspension was left to react under stirring for 24 h. The product was obtained after 10 min centrifugation at 5000 RPM (14.6 rotor radius, 2880 g RCF) and washing process with THF to remove unreacted PpIX. Finally, LuAG:$Pr^{3+}$@$SiO_2$-PpIX nanoparticles were dried in air at 40 °C.

### 2.2. Characterization

#### 2.2.1. Singlet Oxygen Generation

APF (aminophenyl fluorescein; Invitrogen™) commercial probe was used for the singlet oxygen detection. 100 µL of APF was added into 2 mL of ethanol suspension containing 15 mg of LuAG:$Pr^{3+}$@$SiO_2$-PpIX nanoparticles. X-ray tube with Cu anode (voltage 40 kV, current 30 mA, average wavelength $K_{\alpha 1,2}$ 0.15418 nm) was used for irradiation. Samples were irradiated in polypropylene cuvette for 1 h and 2 h. Because APF probe reacts both with $^1O_2$ and OH radicals [39], the measurement with addition of 2.5 mL $10{\cdot}10^{-3}$ mol/L ethanolic solution of $NaN_3$ (≥99.5%, Sigma-Aldrich) as a $^1O_2$-quencher was performed. Subsequently, their photoluminescence spectra were measured in a quartz cuvette.