

STUDYING THE CHROMATIC RESPONSES OF AuNPs EMBEDMENT INTO THE OPAL PHOTONIC CRYSTALS USING THE ALEXA 594 FLUORESCENT DYE

Pham Hong Phong*, Vu Thi Thu Ha

*Institute of Chemistry, Vietnam Academy of Science and Technology,
18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

*Email: phphong@ich.vast.vn

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Abstract. In this study, the chromatic responses of opal photonic crystals (PhCs) based on SiO₂ microspheres fabricated by self-assembly followed by coating polyethylene glycol diacrylate (PEGDA/SiO₂) were investigated. In the presence of Alexa@594 fluorescent dye, the fluorescent intensity of PEGDA/SiO₂ and PEGDA/SiO₂-AuNPs was enhanced 2.2-fold and 3.0-fold, respectively, compared with that of the SiO₂ sample. This was elucidated by investigating the reflection spectra. The obtained stopband clearly showed a red-shift of the PEGDA/SiO₂ about 70 nm (at 623 nm) which could overlap the emission wavelength of the fluorescent dye (617 nm). Plasmon resonance was also achieved when this chromatic response became more significant with the embedment of AuNPs into PEGDA/SiO₂. These obtained results indicated that the material prepared by self-assembly of microsphere SiO₂ coated with PEGDA showed a chromatic response of a PhC. Specially, with the presence of AuNPs in PEGDA, this behavior of the PhC became more significant. A such material can be helpful and promising for further modification to apply to the field of chromatically optical sensing.

Keywords: photonic crystals, PEGDA/SiO₂, fluorescent dye.

Classification numbers: 2.1.1, 2.5.3.

1. INTRODUCTION

In nature, opal photonic crystals (PhCs) can be observed in opal gemstones, or insects such as butterflies, bird feathers and butterfly wings [1]. PhCs are periodic microstructured materials that affect the motion of photons in much the same way that ionic lattice affects electrons in solids, and create iridescent color in eyes. This is because the dielectric constant of PhCs is periodically modulated on a length scale comparable to a desired wavelength. The variation in dielectric function affects the propagation of photons due to the interaction of light with the periodic or random architecture of these materials, leading to color changes depending on the structure of materials. Therefore, there is a band over which photons can not flow, known as a photonic band gap (PBG), which depends on the dielectric media, the refractive index and lattice topology within the PhCs. This characteristic is only observed in PhCs and the potential ability to tune its position to suit specific frequency, and is attractive to many studies in the field of sensing. Thus,

according to Bragg's law, the color observed in the PhCs must be relative to the microstructural periodic features. This means that the detection of a specific tracer based on the colour change of the PhC due to changes of the effective refractive index can be observed by naked eyes. It is due to the fact that PhCs couples with responsive materials and proper functionalization are able not only to select, but also to visualize, by means of the variation of the structural color. Therefore, choices of responsive materials pave the way to the development in the field of sensing.

In order to obtain such materials, several methods such as lithographicology [2, 3] and mask technique [4] have been studied. These routes are well suited to the production of three-dimensional structures with sub-micrometer periodicity. Other routes are more popular approaches such as the formation of artificial opals through the self-assembly of colloidal spheres of either silica or different polymers [5, 6], or the formation of 3D PhCs with structural color fabricated on soft polystyrene [7]. The advantage of the self-assembly method is that a vast variety of materials which lend themselves to multiple purposes can be produced at low cost, such as templates for the formation of 3D PCs in the form of inverse opals, which have a sufficiently high reflective index.

In addition, different kinds of materials with complex dielectric constant, such as gold, silver or metal oxides have also been introduced into the PhCs for supporting both localized and propagating surface plasmon mode. The introduction of these metals has been implemented by different techniques, such as deposition of silver on the PhCs surface by e-beam to form a silver film over nanosphere [8], in-situ reduction of polystyrene PhCs doped with Rhodamine B dye in solution containing gold chloride HAuCl_4 with the presence of reductants [9], or mixing with polystyrene microsphere suspensions used for opal growth [10]. After infiltration of metal nanoparticles into the self-assembled opals, the hybrid metal-dielectric structures are used to achieve the diversion of light efficiency from their incident direction [11, 12] that can support the identification of various compounds [13, 14]. With an easy route and fast fabrication, the self-assembly method has perhaps gained a lot of attention for these materials over the years and continues to be well-received today, because it has not been studied extensively.

In the present study, the chromatic response of PhCs based on PEGDA/ SiO_2 containing AuNPs fabricated by the self-assembly method was investigated. The chromatic response of this material is a proper behavior to evaluate its potential applications in the field of chromatically optical sensors, due to the creation of appealing photonic stopband of the PhCs. For this aim, the Alexa Fluor 594 fluorescent dye, a bright, stable fluorophore emitting into the red range of the color spectrum, is used because the dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. Therefore, such dye plays an important role in conjugating antibodies, peptides or tracers for fabrication of biosensors with fast detection, reversibility and low cost in the field of sensing that is a target for further studies.

2. MATERIALS AND METHODS

2.1. Materials

Tetraethylorthosilicate ($\text{C}_8\text{H}_{20}\text{O}_4\text{Si}$ -TEOS) 99 %, ethanol ($\text{C}_2\text{H}_5\text{OH}$) 99.7 %, and ammonium hydroxide (NH_4OH) 25 % were provided by Merck. All these precursors with high purity were used without further purification. Poly(ethylene glycol) diacrylate (PEGDA) ($M_n = 250$), 2-hydroxy-2-methylpropiophenone 97 % (*Irgacure 1173*), buffered oxide etchant (BOE) and ethanol 95 % were supplied by Sigma-Aldrich. *Irgacure 1173*, a photoinitiator, was used in

radiation curing in the polymerization of PEGDA. AuNPs with a size of 30 nm and Alexa Fluor® 594 fluorescent dye were supplied by Sigma-Aldrich.

2.2. Protocol for fabrication of opalphotonic crystal with AuNPs embedment

Initially, non-functionalized silica (SiO₂) microspheres were prepared by hydrolysis of tetraethoxy silane (TEOS) in a desired medium with the Stöber method as described in the literature [15]. Briefly, the preparation of SiO₂ was performed with a mixture of 2.0 mL of ethanol and distilled water with a proper volume ratio. The solution was mixed in the presence of NH₄OH under stirring conditions at room temperature. Next, 0.45 mL of TEOS was slowly added to this mixture with slow stirring. The hydrolysis reaction was carried out for 4 hours to complete.

The fabrication of PhCs was performed as follows: 250 µL of silica sol suspension was put into a small tube for centrifugation to remove the liquid medium. Before dropping silica, a microscope slide was covered with a hydrophobic thin layer, and it was dried for one day at room temperature. A mixture of 90 wt.% PEGDA and 10 wt.% 2-hydroxy-2-methylpropiophenone was then added to the dried SiO₂ for several minutes and exposed under UV light for polymerization.

For the purpose of AuNPs embedment, 10 µL of AuNPs solution was mixed with 250 µL of silica sol suspension, which was centrifuged before use. This mixture was then used following the same procedures as described above to obtain PEGDA/SiO₂-AuNPs.

To use the Fluor® 594 dye, an accurate amount of this dye at 1.0 mg/mL was dropped onto the control PEGDA/SiO₂ surface, and dried in air before use.

2.3. Instruments

Scanning electron microscopy (SEM, Hitachi S-4800, acceleration of 15-20 Kev, working electrode distance of 4-5 mm) was used to characterize the surface morphology of SiO₂ spheres and energy-dispersive spectroscopy (EDS). The spectrometer processor (Ocean Optics QE Pro-FL), which has a wavelength range of 350 nm ÷ 1100 nm, in conjunction with a halogen light source (Ocean Optics HL-2000) was used to measure the reflection. Fluorescent images of the samples were measured using a fluorescent microscope (BX51, Olympus), while the fluorescence intensity was analyzed using Image J software.

3. RESULTS AND DISCUSSION

3.1. Measurement results obtained by SEM method

The surface morphology of the PhCs fabricated by the self-assembly method is representatively presented in Figure 1(a). The SEM image shows bright circles, corresponding to SiO₂ spherical particles which are coated with a PEGDA thin film. As seen in the top later, the self-assembly of SiO₂ spherical particles creates an ordered structure on a large scale (5 µm), allowing the organization of SiO₂ spheres in a two-dimensional hexagonal array layer with a diameter of 250 nm ± 10 % (see the inset). A few narrow interstitial defects can also be observed. These interstices are resulted from an impurity located at an interstitial site or one of the lattice atoms being in an interstitial position instead of being at this lattice position [16]. Figure 1(b) shows white spots, corresponding to the AuNPs embedded into PEGDA/SiO₂. As

can be seen in the image, the AuNPs are scattered on the surface of the SiO₂ spheres. The size of AuNPs is estimated to be in a range of 30 nm ± 15 %.

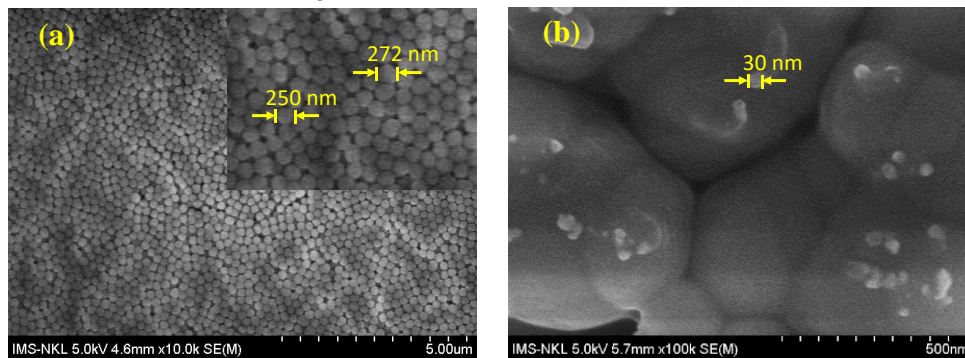


Figure 1. Scanning electron micrographs (SEMs) of PEGDA/SiO₂ (a), the inset: the lower left corner of the image was zoomed in; and of AuNPs embedded into PEGDA/SiO₂ (b).

3.2. Measurement results obtained by EDS method

Figure 2 shows the energy-dispersive spectroscopy (EDS) of AuNPs embedded into PEGDA/SiO₂. In this figure, the existence of AuNPs is indicated by the peak appearing at 2.2 keV, which is a typical absorption peak of metallic Au [17]. While another sharp peak appears at 1.74 keV corresponding to the presence of Si element [18]. Besides that, the presence of O and C elements can also be seen in the image due to the presence of PEGDA in the sample. The component elements in the material are also shown in the table with the percentage of Au reaching 0.12 wt.%. These obtained results are clear evidences for the embedment of AuNPs into PEGDA/SiO₂, denoted as PEGDA/SiO₂-AuNPs.

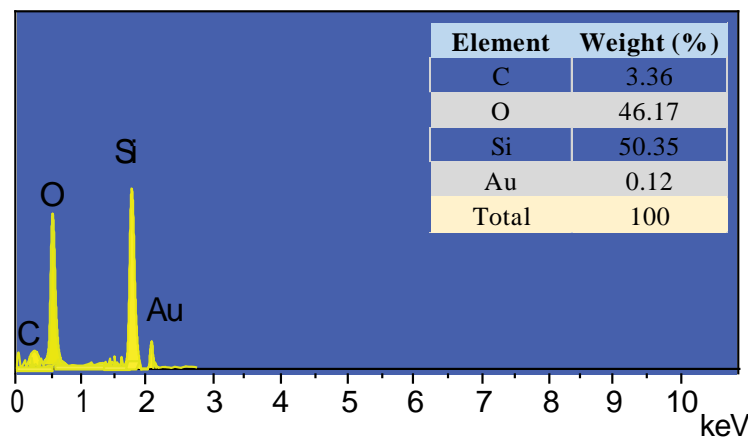


Figure 2. EDS of the PEGDA/SiO₂-AuNPs.

3.3. Results obtained from chromatic responses

The role of PhCs and AuNPs embedment in PEGDA/SiO₂ in enhancing chromatic response compared to the SiO₂ sample is shown in Figure 3. In the presence of the Fluor@ 594 fluorescent dye, as seen in these photographs, the brightness tends to increase for PEGDA/SiO₂ and PEGDA/SiO₂-AuNPs, indicating that the fluorescence intensity is enhanced for the

formation of PEGDA/SiO₂, and specially, for the presence of AuNPs in PEGDA/SiO₂. Image J software was used for image processing to compare the color of the SiO₂ sample and the color of PEGDA/SiO₂ and PEGDA/SiO₂-AuNPs. The obtained results presented in Figure 4 indicate a significant enhancement of the fluorescence intensity of PEGDA/SiO₂ and PEGDA/SiO₂-AuNPs with error bars, estimated from different sites (n) = 3. This figure shows a 2.2-fold and a 3.0-fold increase in fluorescence intensity for the cases of PEGDA/SiO₂ and PEGDA/SiO₂-AuNPs, respectively.

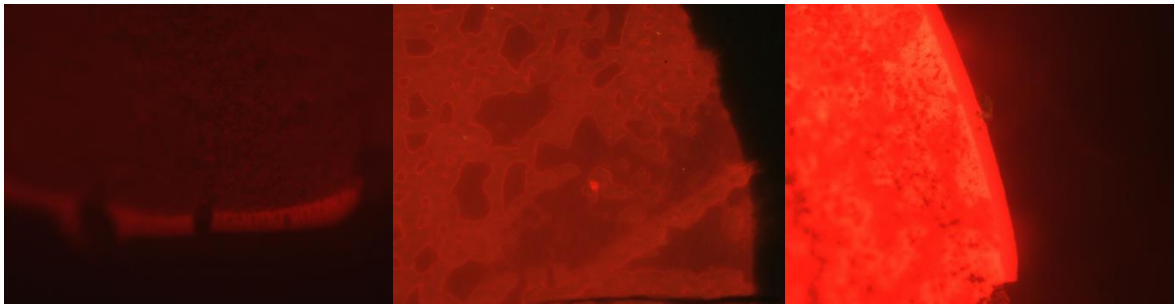


Figure 3. Photographs of SiO₂ (left), PEGDA/SiO₂ (middle), and PEGDA/SiO₂-AuNP (right) were dyed with Alexa Fluor@ 594.

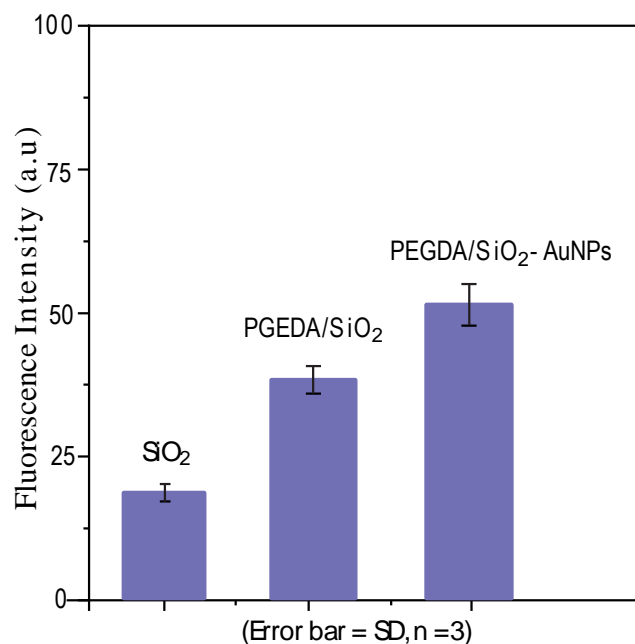


Figure 4. Diagram of the fluorescence intensity variation of SiO₂, PEGDA/SiO₂, and PEGDA/SiO₂-AuNPs were dyed with Alexa Fluor@ 594.

3.4. Discussion on chromatic responses

These obtained chromatic responses indicate that the PEGDA/SiO₂ fabricated by self-assembly behaves as a photonic crystal. Particularly, the fabricated PEGDA/SiO₂ material consists of a two-dimensional (2D) periodic layer of SiO₂ microspheres, coated with a PEGDA thin film. Such structure of PEGDA/SiO₂ possesses a periodic layer with two different reflective

indexes and lattice constant between SiO₂ spheres on the order of visible light's wavelength. An outstanding strong point of PhCs is the formation of a photonic band gap or "pseudo gap" due to the constructive interference of the light by the structure of the PhCs materials [19], leading to a certain wavelength range of light that cannot exist. When the reflective index of the dielectric material is sufficiently large, a complete photonic band gap is formed in which Bragg diffractions inhibit a wavelength range from propagating through the PhCs overall direction. Thus, the color of such materials is independent of electronic absorption, instead, the color is dependent on the reflective index and the periodicity of materials that is comparable to the wavelength of visible light. The relation between the wavelength of diffraction and reflective index of materials is described by Bragg's Law [20]

$$\lambda = 1.633 \times d \times n \quad (1)$$

where: λ : wavelength of the diffraction peak (nm); d : center-to-center distance between two; neighbouring mesopores (nm); n : the average reflective index of the materials. (Herein, the values of SiO₂ and PEGDA are 1.378 and 1.463, respectively, according to the specification of the products.)

Since then, in order to clearly interpret the enhancement of chromatic responses of the PEGDA/SiO₂ and PEGDA/SiO₂-AuNPs, studies of the reflection of these materials have been carried out. This is because studies of the reflection and PBG of PhCs relates to color performance. In addition, the intensity of a reflected wave can also reflect the chromaticity of a material. Thus, studies of the interrelationship between fluorescence and band-gap of a PhC can interpret the improvement of chromatic response of crystal devices [21]. In Figure 5, the comparison of the reflection spectra of samples are shown. The reflection spectra of the SiO₂ sample shows a peak at 556 nm, corresponding to its stopband. The value for the fabricated material is slightly different from the theoretical value, calculated by the Bragg's equation to be 562 nm.

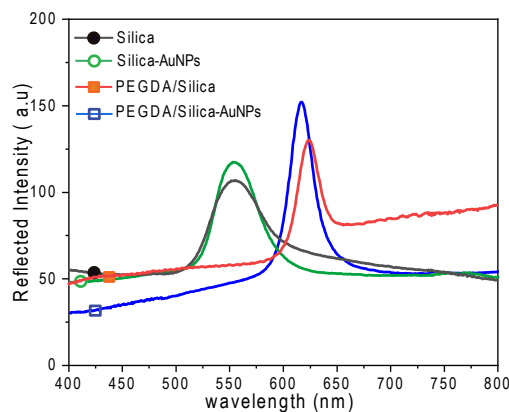


Figure 5. Reflection spectra for SiO₂ self-assembling (●), SiO₂-AuNPs (○), PEGDA/SiO₂ (■), and PEGDA/SiO₂-AuNPs (□).

Such difference in the values of stopband for the SiO₂ material is due to the deviation between the real size of SiO₂ spherical particles and the average size estimated by SEM image (250 nm ± 10 %). Following the equation (1) with a stopband value of 556 nm, the size of the prepared SiO₂ particles is estimated to be 247 nm. Hence, the size of the SiO₂ spheres calculated from these diffraction spectra is consistent with that estimated by SEM.

Regarding the color of the samples, the obtained center of the stopband of the SiO₂ sample is far from 617 nm, which is the emission wavelength of the Fluor@594 fluorescent dye. Thus, there is no overlap of wavelengths in this case, leading to the red color for the SiO₂ sample that might not be clearly observed. When AuNPs are embedded into SiO₂ spherical particles, the stopband of the SiO₂-AuNPs sample is kept almost constant, but the reflected intensity is slightly higher, owing to the plasmon resonance effect. But when the SiO₂ spherical particles are coated with PEGDA, it increases the value of the reflective index of this material, leading to a red-shift of the stopband to 625 nm. This is a reason for the red color of PEGDA/SiO₂ to become brighter. When AuNPs are embedded into PEGDA/SiO₂, the stopband is caused to shift slightly to a lower wavelength at 623 nm. This value is closer to the emission wavelength of the Fluor@594 fluorescent dye, leading to a greater overlap between the two stopbands. Thus, this overlap leads to a local resonance mode for the emission of light. Specially, the presence of AuNPs also induces plasmon resonance. These reasons make the fluorescence intensity of PEGDA/SiO₂-AuNPs to be remarkably enhanced. Therefore, the results obtained from the studies on reflection spectra are in good agreement with the chromatic responses as shown in the photographs presented above.

In addition, as seen in this figure, the peak at 613 nm of the PEGDA/SiO₂-AuNPs becomes narrower as indicated by the full width at half maximum (FWHM). The decrease in FWHM of this peak compared with that of the peak at 556 nm indicates the higher quality of PEGDA/SiO₂-AuNPs. This is because the quality (Q) parameter is inversely proportional to the FWHM [22]. Therefore, the increase in value of Q indicates the role of PEGDA in reducing nanocavities in the obtained PhC generated from the fabrication steps.

Regarding the use of fluorescent dyes for improving the fluorescence intensity of PhCs, many studies have also demonstrated the successful preparation of PhCs by self-assembly of colloidal crystals [23], the ability of PhCs for slowing photons [24], the stability of dye attachment, and the relationship between enhanced fluorescence intensity and pseudo bandgap position relative to a fluorophore fluorescence peak [25]. In this context, our study demonstrates for the first time the use of the Fluor @594 dye infiltrated onto the PhCs of PEGDA/SiO₂-AuNPs for improving the fluorescent intensity. This chromatic response of the PhCs can be applied to the field of optical sensing.

4. CONCLUSIONS

In this study, the chromatic response of the opal photonic crystals based on SiO₂ fabricated by self-assembly technique and coated with poly-ethyleneglycol diacrylate (PEGDA/SiO₂) was investigated in the presence of Alexa@594 fluorescent dye. The obtained results indicated that PEGDA/SiO₂ induced a photonic red-shift stopband, which was closer to the emission wavelength of the dye, causing an overlap which resulted in an increase in the fluorescence intensity. This chromatic response became more significantly with the embedment of AuNPs due to the surface plasmon resonance. Thus, these obtained results indicated that PEGDA/SiO₂-AuNPs fabricated by self-assembly method could be used as a photonic crystal for further modification to apply to the field of chromatically optical sensing.

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CRedit authorship contribution statement. Pham Hong Phong: Methodology, Investigation. Author 2: Formal analysis, Supervision.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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