Suppression of Melanin Synthesis by Europium Doped Cerium Oxide Nanomaterials

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I. Introduction

Cerium (IV) oxide, CeO_2, is of significant research interest due to its dual Ce^{3+}/Ce^{4+} oxidation states. Addition of trivalent ions, such as Ce^{3+}, or Eu^{3+}, into the CeO_2 lattice results in a metal oxide with a high number of oxygen vacancies.[1] The oxygen vacancies, along with the energetically available transition between the two states, allow CeO_2 to act as an antioxidant as well as mimic several enzymes such as catalase and superoxide dismutase.[2] This activity, along with fluorescence from Eu^{3+} makes Eu^{3+}-doped CeO_2 ($EuCeO_2$) nanomaterials potentially useful as probes in biological systems.

The dark brown pigment eumelanin, is synthesized by oxidation and oligomerization of tyrosine, or by the auto-oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA). Eumelanin has strong UV-VIS absorption and radical scavenging. However, some research suggests melanin may be degraded with UVA light in the right conditions, producing high-energy unstable fragments, leading to damaged DNA and leading to cancer. Therefore, it is of interest to control the synthesis of eumelanin, and the production of these fragments.[3]

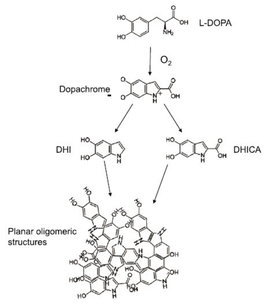

Figure 1. Oxidation and oligomerization of L-DOPA to synthesize one suggested structure of eumelanin[4]

II. Methods and Materials

Nanomaterial Synthesis

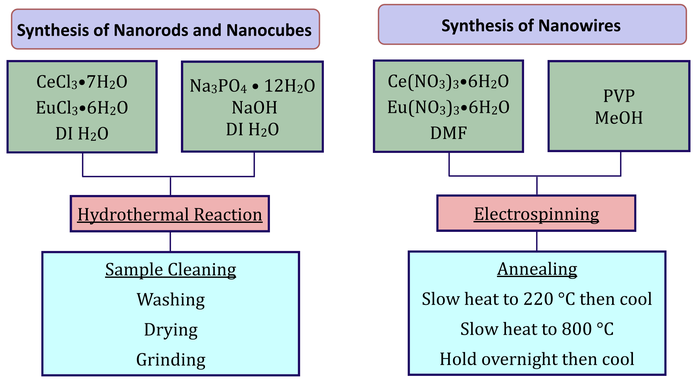

Figure 2. Synthetic route for fabrication of Eu-CeO_2 nanorods, nanocubes, and nanowires

$EuCeO_2$ nanorods were annealed in a tube furnace for 2 hr at 700°C then cooled to room temp.

Fluorescence imaging was recorded with a Nikon fluorescent microscope with an excitation wavelength of 370nm, and fluorescence spectra were recorded by an Ocean Optics spectrometer with an acquisition time of 200ms.

Eumelanin Studies

All eumelanin experiments were performed in water with pH of 10, adjusted using NH_4OH, unless otherwise indicated. $EuCeO_2$ nanomaterials were sonicated for 3 minutes using Branson Sonifier 450 sonication probe, 20 % power output and 60% cycling, prior to mixing with L-DOPA stock sol'n.

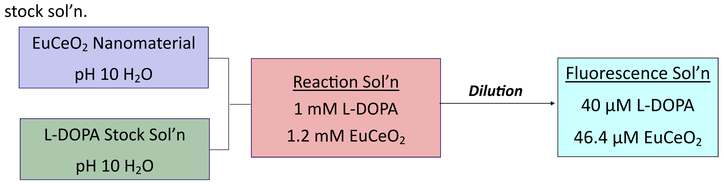

Figure 3. Synthesis and fluorescence of eumelanin. Fluorescence λ_{ex}=375 nm and λ_{em}=471 nm, with emission measured from 400 nm to 600 nm.

III. Results

Crystalline nanorods, nanocubes, and nanowires were all produced with the desired morphology, as shown in Figure 3. The nanorods and nanocubes did form dense aggregates along with the desired morphology, as shown in Figure 3. The %Eu content was controlled by manipulation of the Eu^{3+} precursor concentration.

(1) Nanomaterial Characterization-TEM

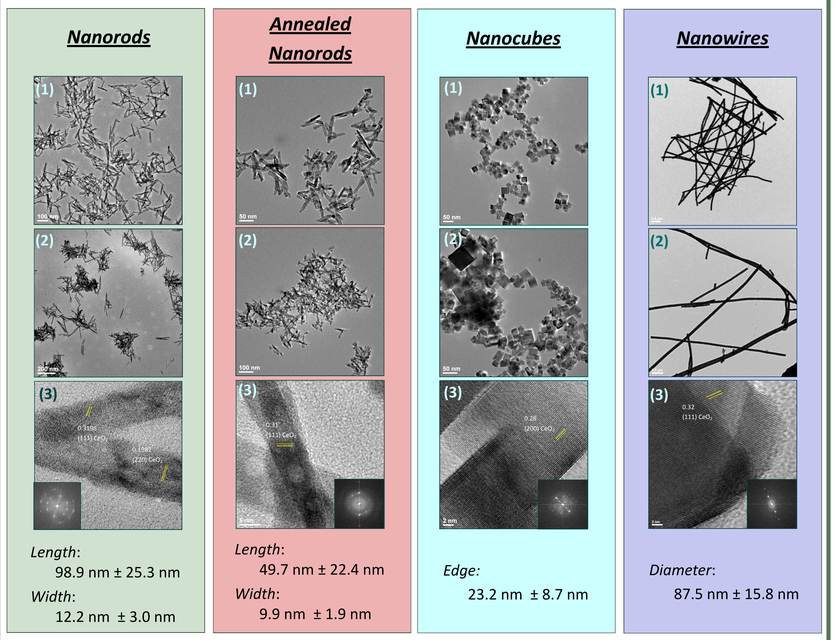

Figure 3: TEM imaging of $EuCeO_2$ nanomaterials morphology of (1) nanorods, cubes or nanowires, (2) aggregates (if applicable) and (3) HR-TEM showing lattice spacing and crystal plane assignments

(2) Nanomaterial Characterization-Fluorescence

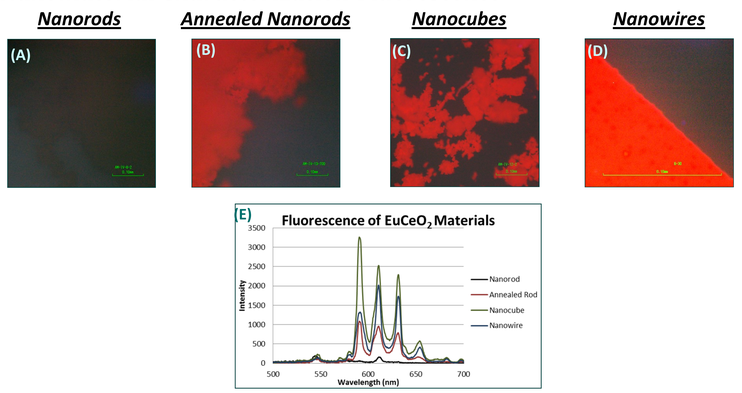

Figure 4. TEM images showing fluorescence of (a-d) $EuCeO_2$ nanomaterials, and (e) fluorescence spectra of all four nanomaterials.

(3) Eumelanin Studies

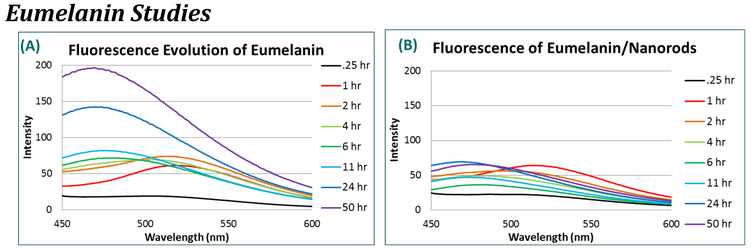

Figure 5. Progression of fluorescence associated with formation of (a) eumelanin and (b) eumelanin in the presence of $EuCeO_2$ nanorods.

III. Results (cont.)

Fluorescence λ_{ex}=375 nm and λ_{em}=471 nm, and reported as normalized to the sample's emission at 15 minutes. Control samples are L-DOPA in the absence of any nanomaterial.

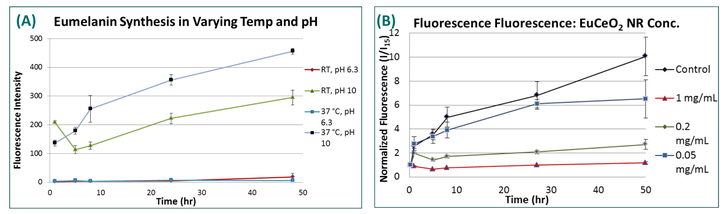

Figure 6. (a) Raw fluorescence intensity at 471 nm, showing influence of pH and temperature on the production of eumelanin, and (b) normalized fluorescence showing impact of nanorod concentration.

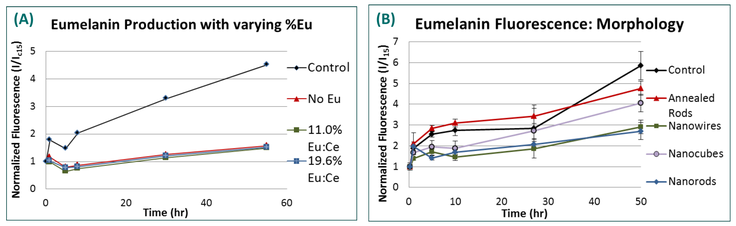

Figure 7. Plots normalized fluorescence showing the influence of (a) Eu^{3+} concentration, and (b) $EuCeO_2$ nanomaterial morphology on synthesis of eumelanin.

IV. Conclusions

- $EuCeO_2$ nanorods and nanocubes were synthesized by a hydrothermal route. Fluorescence in the nanorods was activated by annealing to 700 °C.
- $EuCeO_2$ nanowires were synthesized by an electrospinning and annealing route.
- L-DOPA oxidizes over several days, as tracked by fluorescence between 470 nm and 540 nm.
- Increased pH is necessary for the production of eumelanin. Increasing the reaction temperature increases the reaction rate, but is not necessary for production of the pigment.
- The presence of $EuCeO_2$ nanomaterials significantly suppresses the fluorescence associated with eumelanin. Nanorod concentrations down to 0.2 mg/mL showed significant eumelanin suppression.
- The Eu^{3+} concentration in nanorods did not significantly impact eumelanin fluorescence
- Unannealed nanorods and nanowires showed comparable eumelanin suppression, with nanocubes and annealed nanorods showing weaker suppression.
- Future work will focus on analysis of mechanism behind eumelanin fluorescence suppression, fluorescence lifetime measurements, and analysis of potential $EuCeO_2$/eumelanin composite properties.

V. Literature Cited

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