Developing an Oxygen Detection Device for a Microfluidic Hypoxia Chamber
Experimental Protocol and Procedures
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EXPERIMENTAL PROCEDURE FOR CREATION OF STANDARIZED CURVE AND OPTIMIZATION OF THIN-FILM SENSOR

1. Prepare PtOEPK matrix at different volume ratios of matrix: PtOEPK
    a. Test to find optimal ratio
    b. Dissolve PS pellets in toluene solution (7% w/w ideal, test dramatically above and below)
        i. Possible tests: 17% w/w and 1% w/w
        ii. GOAL: Find optimal w/w percent of encapsualtion matrix.
    c. PtOEPK dye added to matrix
        i. Test conditions of 0.1mg: 1mL, 1mg: 1mL, and 10mg: 1mL
        ii. GOAL: Find optimal ratio of luminescent material in encapsulation matrix
2. Transfer ~30 μL of PtOEPK matrix into each well of the 96-well plate
    a. Need to test to determine effective volume needed to completely cover bottom of well
    b. Films will need to be as thin as possible to optimize response time
        i. If time, attempt to test films of varying thickness
    c. Spin-state wells to pull all material to bottom of wells
3. Allow the PtOEPK matrix to stand in wells for 24hrs at room temperature in dark for gelation and drying
    a. NOTE: may require longer time to dry
        i. Need to determine time needed for films to gel
4. Vary concentration of oxygen in 96-well plates (percentage of oxygen, v/v) by manipulating the flow rate of oxygen and nitrogen flowing into the microwell containing the oxygen sensing film
    a. Vary concentrations from 1% to 25% if possible
5. The quenching response is determined by measuring the luminescence intensity of films under no oxygen and a fully oxygenated solution
6. Plot quenching response versus volume ratio
7. Determine Stern-Volmer plots by measuring luminescence intensity under various gaseous oxygen or dissolved oxygen concentrations.

**At this point in the design photostability and leaching effects will not be tested as they have thoroughly be proved by other labs and previous work

HYPOTHESIS:

From previous research and findings, we believe that the thin-film sensor will work most efficiently with 7% w/w PS in toluene solution and a 1mg: 1mL PtOEPK in encapsulation matrix. Additionally, as oxygen concentration is increased intensity of the luminescent material should decline as in the presence of oxygen oxidation occurs with Pt- causing luminescent properties to decline.

EXPERIMENTAL DESIGN

Test will need to be performed multiple times to demonstrate high reproducibility of the thin-film sensors

- Test 1A:
  - 1% w/w PS in toluene solution
  - 0.1 mg: 1mL ratio of PtOEPK in encapsulation matrix
- Test 1B:
  - 1% w/w PS in toluene solution
  - 1 mg: 1mL ration of PtOEPK in encapsulation matrix
- Test 1C:
  - 1% w/w PS in toluene solution
  - 10 mg: 1mL ration of PtOEPK in encapsulation matrix
- Test 2A:
  - 7% w/w PS in toluene solution
  - 0.1 mg: 1mL ratio of PtOEPK in encapsulation matrix
- Test 2B:
  - 7% w/w PS in toluene solution
  - 1 mg: 1mL ration of PtOEPK in encapsulation matrix
- Test 2C:
  - 7% w/w PS in toluene solution
  - 10 mg: 1mL ration of PtOEPK in encapsulation matrix
- Test 3A:
  - 17% w/w PS in toluene solution
  - 0.1 mg: 1mL ratio of PtOEPK in encapsulation matrix
- Test 3B:
  - 17% w/w PS in toluene solution
  - 1 mg: 1mL ration of PtOEPK in encapsulation matrix
- Test 3C:
  - 17% w/w PS in toluene solution
  - 10 mg: 1mL ration of PtOEPK in encapsulation matrix

**For all test conditions, quenching rate, max and min intensity, and Stern-Volmer rates will be determined

**Due to time constraints, team will begin with Test 2, as it is hypothesized to be the optimal conditions.

ANALYSIS

- Optimal composition of thin-film sensor should provide highest sensitivity
    - Sensitivity can be expresses by quenching response and quenching ratio (I_0/I_{100}, where I_0 is luminescence intensity at 100% N_2 and I_{100} is luminescent intensity at 100% O_2)
- Films will need to be as thin as possible to optimize response time to excitation
- Test will need to be performed multiple times to demonstrate high reproducibility of the thin-film sensors