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14. ABSTRACT

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RPPR Final Report

as of 02-Feb-2022

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Agreement Number: W911NF-16-1-0455

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Final Report for Period Beginning 10-Aug-2016 and Ending 22-May-2021

Title: 9.1 Materials by Design: Molecular Contributions to Coloration in Cephalopod Chromatophores for Bio-inspired Photonic Systems

Begin Performance Period: 10-Aug-2016

End Performance Period: 22-May-2021

Report Term: 0-Other

Submitted By: Leila Deravi

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STEM Degrees: 1

STEM Participants:

Major Goals: Major Goals: This fundamental (6.1) innovative research investigates next generation, sustainable coatings that address PP-13-12-03: "green" pigments that provide both visible and infrared (IR) coloration in polymers, without using chromium-based pigments, and responsive, multi-functional surface coatings that include tunable pigments with low ESOH risk. CCDC-SC and Northeastern University have partnered to build on earlier informal collaboration under a CCDC-SC 6.1 nano-enhanced scattering program. In thin films containing chromatophore pigment granules (diameter ~ 500 nm), we observed large (> 40%) forward scattering of visible and near-IR light, providing clues on how to obtain ideal coloration (e.g., bright and spectrally-pure reflectivity) and tunability when combined with the underlying cephalopod iridophores and leucophore organs. In this EQBR work, our major goals are to expand on these exciting findings by:

- 1- Measure/model back- and forward-scattering of cephalopod granules
- 2- Compare to control samples for scattering: plasmonic nanoparticles
- 3- Determine effect of thickness, particle distribution, and reflector on scattering/absorption; Deliverable: create and test molecular-based model describing bio-optics and color richness in cephalopods
- 4- Investigate tunability of color in response to pH and electrochemical triggers
- 5- Investigate tunability of color in response to optical triggers
- 6- New design/synthesis of samples (Deliverable) to improve tunability
- 7- Transition to fiber platform (Deliverable: scattering fibers)
- 8- Demonstrate tunability of color in response to trigger(s) in films

Accomplishments: 1. Fabricated films comprising a distribution of squid pigment granules with multiple thicknesses. Monolayer and multilayer films were designed to represent the dynamic states (fully expanded to punctate forms) of the chromatophore. Films were analyzed using total integrated diffuse scattering (TIDS at CCDC-SC), indicating roles of forward and backward scattering on perceived visible color.

a. We have recently replicated these films and analyzed with ellipsometry

b. Here, 18 cm × 18 cm glass slides were sonicated for 30 min into isopropanol, ethanol, and acetone prior to use. Once dried, a 8.3 wt/vol solution of PAA (pH 3.5) was dispensed on the glass surface and spun for 1 min at 2000 rpm to obtain a uniform coating using a G3P-8 Spin Coater. Next, a granule-water suspension (concentration was varied from 0.16 to 2.45 mg/mL in water) was drop-casted directly onto the PAA-coated glass surface and dried at 70 °C for 1 h, until the films were dried. For the pigment-extracted granules and the pigment only films, a similar procedure was used to make the films.

c. Results show a robust manufacturing procedure that can generate robust films for analysis.

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d. Ellipsometry for n and k measurements of thin films for modeling of the granule mediated coloration. Based on white-light visible-infrared ellipsometric measurements of broadband (n , k) from synthesized smooth films of natural squid xanthommatin pigment, extracted from squid granules and combined with a polymer to prevent aggregation, we have reported broadband indices of refraction (n , k) for xanthommatin pigment from 300 nm to 3.2 micrometers, enabling new classes of optical design that require a full optical model of xanthommatin. We discovered a heretofore-unreported absorption (k) peak around 3 μm , which we attribute to stretching bonds in the natural squid xanthommatin. We also confirmed that n at 1.55 at 589 nm, consistent with our past work. These findings and their discovery and quantification of new optical (visible/infrared) materials physics properties will enable new optical designs using smart, responsive, multifunctional, lightweight biological and bio-inspired materials.

2. Showed proof of concept color change in presence of changes in pH and redox potential.

a. A 1 mM solution of squid extracted and purified pigment was prepared in water and titrated to a starting pH of ~ 2 . The solution was then titrated with NaOH until the pH reached ~ 10 . During each point in the titration, the pH and absorbance of the solution was determined. pH levels were measured using a Fisher Scientific Accumet AP110 pH meter (Fisher Scientific, Waltham, MA). Absorbance was measured using a Spectramax microplate reader.

b. We incorporated pigments into devices that can trigger color switches in response to applied electric/electrochemical potential.

3. Fabrication of synthetic nano/microparticles that mimic squid pigment granules.

a. Synthetic particles are synthesized using an amine-catalyzed precipitation reaction in monosilicic acid to condense SiO₂ nanoparticles (SiO₂ refractive index ~ 1.46 , representing a good "shell" mimetic in the synthetic granules)

Training Opportunities: Postdoctoral research assistant Jason (Zhaungsheng) Lin led the collaboration and facilitated materials transferred between Northeastern and CCDC-SC (Natick, MA)

Results Dissemination: Leila Deravi has presented EQBR based research at the following invited seminars:

- April 2021 Texas A&M, virtual seminar
- April 2021 Purdue University, virtual seminar
- March 2021 Vanderbilt University, virtual seminar
- March 2021 Kalamazo College, virtual seminar
- March 2021 ARL Mini-Workshop on Reconfigurable Materials, virtual
- February 2021 Duke University, virtual seminar
- February 2021 Virginia Tech, virtual seminar
- February 2021 University of Rhode Island, virtual seminar
- November 2020 Materials Research Symposium, Boston, MA, virtual seminar

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Leila F. Deravi, Zhuangsheng Lin, Patrick Sullivan, Cassandra L. Martin. "A naturally-derived and edible photonic crystal color system," U.S. Provisional Patent Application No.: 63/050,728, 2021.

PARTICIPANTS:

Participant Type: PD/PI

Participant: Leila Deravi

Person Months Worked: 1.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)

Participant: Jason (Zhuangsheng) Lin

Person Months Worked: 3.00

Project Contribution:

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Article Title: Color Richness in Cephalopod Chromatophores Originating from High Refractive Index Biomolecules

Authors: Sean R. Dinneen, Richard M. Osgood, Margaret E. Greenslade, Leila F. Deravi

Keywords: squid, chromatophore, refractive index

Abstract: Cephalopods are arguably one of the most photonically sophisticated marine animals, as they can rapidly adapt their dermal color and texture to their surroundings using both structural and pigmentary coloration. Their chromatophore organs facilitate this process, but the molecular mechanism potentiating color change is not well understood. We hypothesize that the pigments, which are localized within nanostructured granules in the chromatophore, enhance the scattering of light within the dermal tissue. To test this, we extracted the phenoxazine-based pigments from the chromatophore and extrapolated their complex refractive index (RI) from experimentally determined real and approximated imaginary portions of the RI. Mie theory was used to calculate the absorbance and scattering cross sections (cm²/particle) across a broad diameter range at $\lambda = 589$ nm. We observed that the pigments were more likely to scatter attenuated light than absorb it and that these characteristics may contribute to

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Article Title: Optical extinction of size-controlled aerosols generated from squid chromatophore pigments

Authors: Sean R. Dinneen, Margaret E. Greenslade, Leila F. Deravi

Keywords: cavity-ring down, squid, chromatophore, granules, aerosols

Abstract: Nanophotonic granules populate the interior of squid *Doryteuthis pealeii* chromatophores, contributing to dermal color richness by selectively absorbing and scattering light. Inspired by the performance of these granules, we fabricated nanostructured aerosols by nebulizing a pigment solution extracted from native squid chromatophores. We determined the extinction of these aerosols using cavity ring-down (CRD) spectroscopy and show how extinction cross-section is dependent on both particle concentration and size. This work not only advances the fundamental knowledge of the optical properties of chromatophore pigments, but also serves as a proof-of-concept method that can be adapted to develop coatings derived from these materials.

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Article Title: Xanthommatin based electrochromic displays inspired by nature

Authors: Amrita Kumar, Thomas L. Williams, Camille A. Martin, Amanda Figueroa Navedo, Leila F. Deravi

Keywords: electrochromic device, spectroelectrochemistry, redox, xanthommatin, poly(3,4-ethylene dioxythiophene)?poly(styrenesulfonate)

Abstract: Color is a signature visual feature in nature; however, the ability to trigger color change in the presence of different environmental stimuli is unique to only a handful of species in the animal kingdom. We exploit the natural color changing properties of the predominant pigment in arthropods and cephalopods - xanthommatin (Xa) - and describe its utility as a new broad-spectrum electrochromic material. To accomplish this goal, we explored the spectroelectrochemical properties of Xa adsorbed to an indium-doped tin oxide (ITO) coated substrate chemically modified with poly(3,4-ethylene dioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS). We identified a synergistic role between PEDOT:PSS and Xa that contributed to its absorption profile which could be modulated across multiple cycles. By varying the ratio of the two-electroactive components, we also altered the perceived visible color of Xa-based devices which cycled from different shades of red to yellow under reducing and o

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Article Title: A bioinspired, photostable UV-filter that protects mammalian cells against UV-induced cellular damage

Authors: Camille A. Martin, Mahboobeh Rezaeeyazdi, Thibault Colombani, Sean R. Dinneen, Amrita Kumar, Sidi

Keywords: xanthommatin, UV filter, cytotoxicity

Abstract: While commercially available sunscreen products are effective at absorbing ultraviolet (UV)-light, recent studies indicate systemic toxicities associated with many traditional chemical and physical UV-filters. We demonstrate the application of xanthommatin, a biochrome present in arthropods and cephalopods, as an alternative chemical UV-filter that is cytocompatible while maintaining its photostability and photoprotective properties.

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Article Title: Biomimetic Colorants and Coatings Designed with Cephalopod-Inspired Nanocomposites

Authors: Camille A. Martin, Zhuangsheng Lin, Amrita Kumar, Sean R. Dinneen, Richard M. Osgood, Leila F. Deravi

Keywords: colorants coatings xanthommatin bioinspired nanoparticles silica

Abstract: Brilliant and dynamic colors in nature have stimulated the design of dyes and pigments with broad applications ranging from electronic displays to apparel. Inspired by the nanostructured pigment granules present in cephalopod chromatophore organs, we describe the design and fabrication of biohybrid colorants containing the cephalopod-specific pigment, xanthommatin (Xa), encased within silica-based nanostructures. We employed a biomimetic approach to encapsulate Xa with amine-terminated polyamidoamine (PAMAM) dendrimer templates, which helped stabilize the pigment during encapsulation. Depending on the concentration of Xa used in the reaction, the resultant biohybrid nanomaterials generated a range of neutral colors of differing hues. When applied as coatings, these colorants can be triggered to change color from yellow/gold to red in the presence of a chemical reducing agent, as we leverage the natural redox-dependent color change of Xa. Altogether, these capabilities demonstrated the

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Partners

I certify that the information in the report is complete and accurate:

Signature: Leila Deravi

Signature Date: 2/1/22 8:23PM

EQBR

Ellipsometric measurement of pigment films: In an effort to elucidate the optical contribution of the pigment to the films fabricated for ellipsometry (**Figure 1**), samples are prepared in increasing concentrations of pigment to PVA matrix. Through this series of samples, a correlation can be drawn between emerging spectral features and the concentration of pigment within the thin film. Solutions for spin-coating are prepared so that the resulting dried film will comprise a certain percent by weight of pigment vs. PVA. 100% pigment films have been attempted in the past, but the pigment tends to aggregate, resulting in films that are rough and therefore not suitable for ellipsometric measurement.

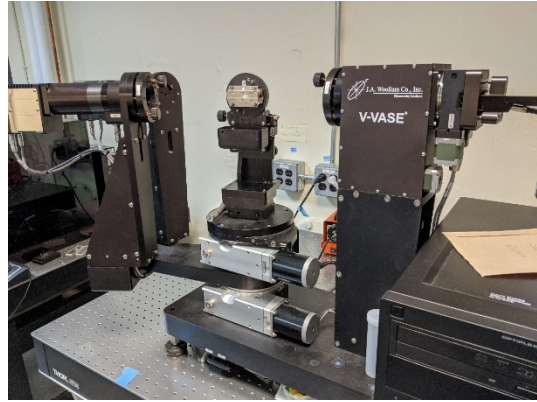


Figure 1. J. A. Woollam V-VASE model ellipsometer used to measure optical properties of pigment thin films

Samples made to date:

- 30% Pigment/PVA
- 40% Pigment/PVA
- 50% Pigment/PVA
- 70% Pigment/PVA
- 80% Pigment/PVA

Results from the 50% Pigment/PVA sample have been shown in previous reports. To extrapolate the contribution of the pigment to the measured film's refractive index, we calculated the pigment's n using the plots of both the 50% pigment/PVA and 100% pigment films.

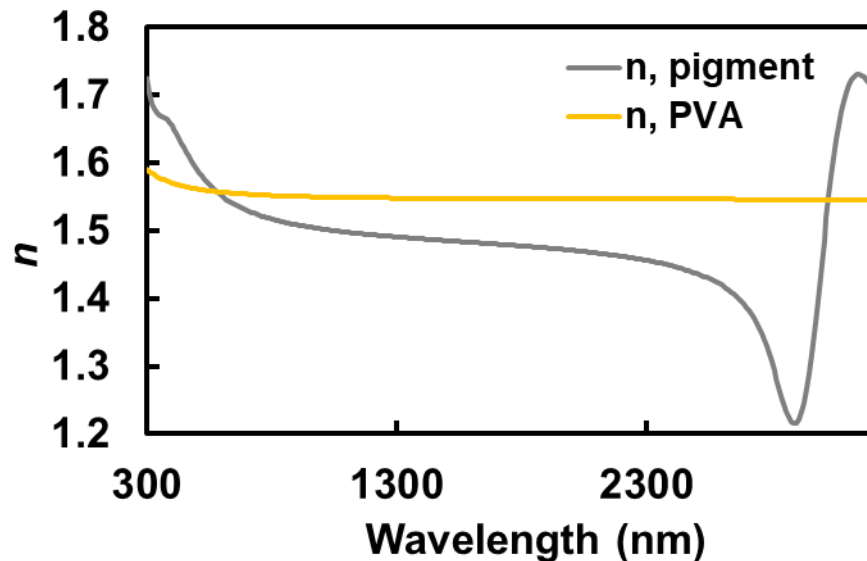


Figure 2. Calculated refractive index, n , vs. wavelength of pigment, extracted from the data of the best-fit graph of n of the 50% pigment/PVA film and the 100% PVA control film

We carried out early-stage analysis of vis and near-IR scattering from chromatophores in films and fibers. In FY19, we believe we were the first to determine quantitatively the optical indices (n , k) of squid pigment over a broad wavelength range, including a discovery of a new infrared absorption peak at 3 nm, probably a molecular stretching resonance, after fabricating smooth films of natural pigments, with 50% pigment by weight, via a new protocol. This is important for designing new optical functionality, including coloration and tunability. In FY19, we illustrated the tunability of Xa via pH and electrochemical triggers, and created synthetic granules, containing encapsulated Xa within silica and Xa coatings on PSMA nanoparticles; the latter were assembled into sub-monolayer arrays for optical scattering analyses. Promising breakthroughs (two publications, discovery of pigment optical properties, demonstrated tunability and synthesized Xa/granules) greatly benefit further R&D on coloration of enhanced, responsive green pigments. Risk is appropriate for high-payoff, far-sighted 6.1 research.

In contrast to earlier studies where fixed wavelength (e.g., laser) measurements were employed, white light ellipsometry was used in this study for broadband determination of the optical indices of the squid xanthommatin pigment. The ellipsometric angles (ψ and δ) were measured at angles of incidence $65^\circ - 75^\circ$, where there is significant contrast between p- and s-reflectivities, and from them n and k were extracted. Ellipsometry required smooth films that couldn't depolarize the signal; otherwise, (n , k) cannot be measured with certainty, since ψ and δ are the Arc Tangent of, respectively, the absolute and imaginary part of the ratio of the p-reflectivity to s-reflectivity. Rough films tended to depolarize the incident light, resulting in a large uncertainty in the ellipsometric measurement.

Xanthommatin pigment was extracted from squids employing acidified methanol, and spincoated in films with, after some trial and error involving other polymers, PVA polymer to retain homogeneity and allow for a smooth film to form via spincoating. Films were smooth and dark-colored. Indices for PVA (k for PVA was expected to be zero in the visible and near-infrared and that was verified to be the case) had to be separately measured from control samples (thickness ~ 570 nm), and extracted from the measurements of the combined polymer-xanthommatin films analytically, using ratio of mixtures for n . Because k in the polymer-xanthommatin films came only from the xanthommatin pigment, the raw measurement gave k directly for the xanthommatin pigment.

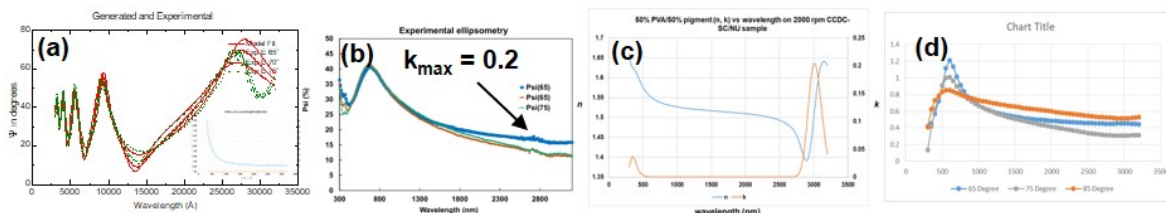


Figure 3 (a) Ellipsometric ψ data at three different angles of incidence vs. wavelength (λ) for 564 nm PVA (fitted n vs. λ in the inset); an analytical model is quantitatively similar. (b) ψ vs. λ for 116 nm PVA(50%)/natural Xa(50%) (c) numerically modeled (n , k) and (d) an analytic model of ψ using the same (n , k) functions

Modeling. The J. A. Woollam software [9] was used to fit the experimental data, but cross-checked with relatively straightforward analytical calculations. The extracted k vs λ spectrum was entirely due to the xanthommatin pigment, since PVA was verified to be non-absorptive in the measured wavelength range. However, to determine n vs. λ of the pigment, we had to factor out the index of refraction of the PVA, using the measurements of total (n , k) of the sample. We discovered a heretofore-unreported peak in k (absorption) peak around 3 μm . This peak was fitted using a Voigt function (convolution of Gaussian and Lorentzian). We attribute this peak to stretching bonds in the natural squid xanthommatin pigment.

Preparation of xanthommatin pigment functionalized particles: Xanthommatin functionalized nanoparticles were synthesized via EDC coupling of xanthommatin pigments onto polystyrene nanoparticles with carboxylic acid functional groups (Figure 3) according to previous protocols with modifications.^{1, 2} Briefly, an aliquot of 120 μL of 5 mg/mL EDC was added into 2 mL 0.125 wt % 200 nm carboxylic acid functionalized polystyrene, and the mixture was stirred at room temperature for 30 min. Then, an aliquot of 500 μL of 2 mg/mL xanthommatin in 0.1M MES buffer (pH 5.5) was added into the mixture and stirred for another 2.5 hours to tether the xanthommatin pigment. The resulting xanthommatin functionalized polystyrene nanoparticles (PS200-XA) was centrifuged at 10,000 g for 5 min and washed using DI water three times. The PS200-XA has a particle size of 198.0 ± 1.6 d.nm, a low polydispersity index of 0.064 ± 0.056 suggesting the particles are monodispersed, and a zeta potential of -23.7 ± 4.66 mV, determined using a dynamic light scattering (DLS) system. The picture of the carboxylic acid functionalized and xanthommatin functionalized particle suspensions was shown in Figure 4. The xanthommatin functionalized particles suspension showed an orange color.

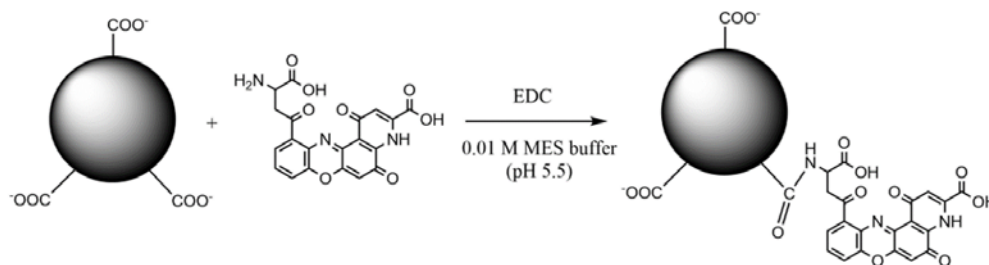


Figure 4. Synthesis scheme of xanthommatin functionalized polystyrene nanoparticles.

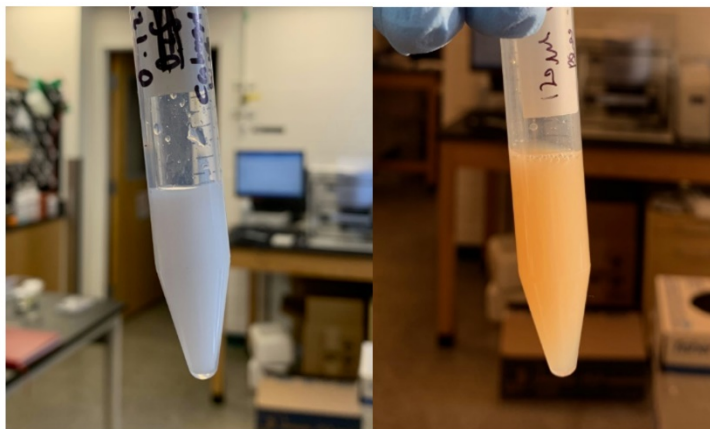


Figure 4. Suspension of carboxylic acid functionalized polystyrene nanoparticles (left) and xanthommatin functionalized polystyrene nanoparticles (right).

Preparation of sub-monolayer film: The sub-monolayer films was prepared by roll-coating according to a previous protocol with modifications (Figure 5).³ Briefly, glass slides and glass vials for roll-coating were cleaned with water, acetone and ethanol by sonication. The glass slides were further cleaned in UV-ozone cleaner for 15 min. Each piece of cleaned glass slide was placed glass slide in 0.01% Polyvinylpyrrolidone (PVP) in ethanol in a glass vial, and rolled for 24 hours to apply PVP coating. The PVP coated films were rinsed with ethanol and dried in a fume hood. Each piece of dried PVP coated film was placed in a 0.0125% PS suspension (control) and a PS200-XA suspension rolled for 24 hours. The particle coated films were dried in a fume hood. A poly(methyl methacrylate) (PMMA) coating was applied onto the particle coated films by spin coating a 0.1% of PMMA in anisole solution to hold the particles in place. The control PS film showed a uniform coating with average interparticle spacing of 468 ± 10 nm ($n=20$) (Figure 6). PS200-XA coated film was not as uniform as the control film. The overall interparticle spacing of the PS200-XA film was larger than the control film. Some area on the glass slide has dense particle coating, while some area on the glass slide has sparse particle coating. This was likely caused by the real particle concentration in the coating suspension being much more diluted than the control suspension due to the loss of particle during the washing steps. Upon application of PMMA coating, the PS particles disappeared. This is likely because the PS particles were dissolved by anisole.

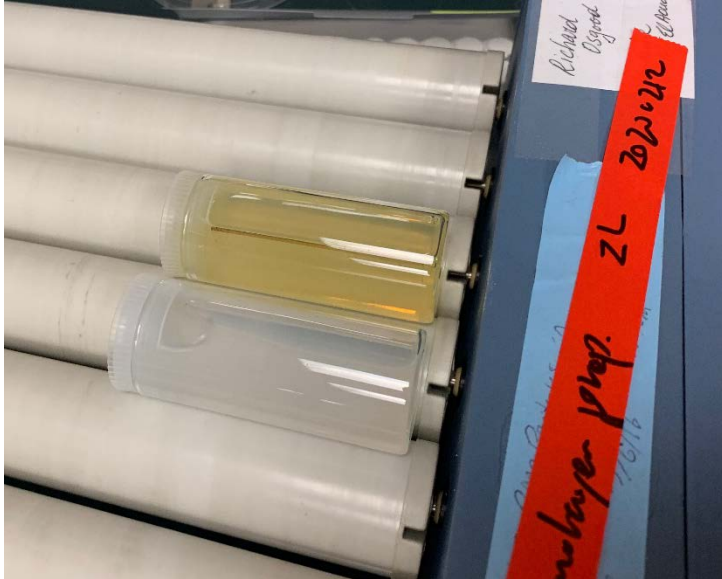


Figure 5. Sub-monolayer film preparation: PS200-XA suspension (top) and PS suspension (bottom).

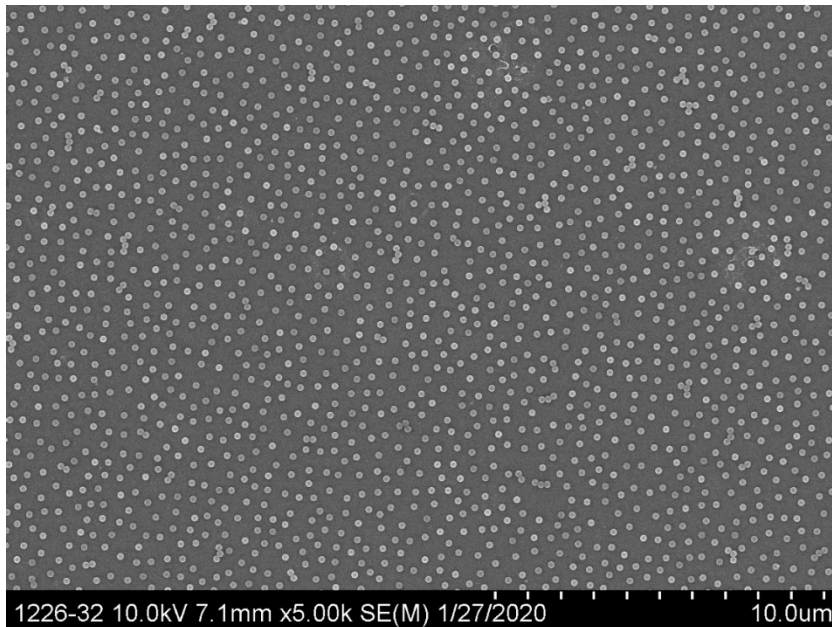


Figure 6. Representative SEM image of control PS sub-monolayer film nanoparticles.

We prepared uniform PS200-XA coated sub-monolayer films by increase the particle concentration of the PS200-XA coating suspension. To hold the coated particles in place, we will find a protective coating formulation to immobilize the coated particles. PMMA and PS has similar thermal and physical properties, and have similar solubility in common solvents. Instead of PMMA coating, we will test spin coating hydrophilic polymer coatings (e.g. poly(acrylic acid), poly(lactic acid)) that are dissolved in solvents that doesn't dissolve PS (e.g. ethanol).

References:

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2. Kumar, A.; Williams, T. L.; Martin, C. A.; Figueroa-Navedo, A. M.; Deravi, L. F., Xanthommatin-Based Electrochromic Displays Inspired by Nature. *ACS Appl Mater Interfaces* **2018**, *10* (49), 43177-43183.
3. Kinnan, M. K.; Kachan, S.; Simmons, C. K.; Chumanov, G., Plasmon coupling in two-dimensional arrays of silver nanoparticles: I. Effect of the dielectric medium. *The Journal of Physical Chemistry C* **2009**, *113* (17), 7079-7084.