

Supporting Information

Synthetic Melanin Nanoparticles for Adsorption of Heavy Metal Ions in Aqueous Environments

Experimental methodology

Materials and Reagents

All reagents for melanin synthesis and metal ions extraction were used as purchased. The 5,6-diacetoxy indole (DAI, >98.0%) was purchased from TCI; the metal salts ($\text{Co}(\text{NO}_3)_2$, NiSO_4 , $\text{CuSO}_4 \cdot 6\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2$, $\text{Pb}(\text{NO}_3)_2$, CdSO_4) with purity greater than 99% were from Sigma Aldrich. Sodium hydroxide pellets (NaOH), Hydrogen peroxide (30 vol% in water), and nitric acid (70%) were from Fisher Scientific. Ethanol was purchased from Decon labs, Inc. Metal ion stock solutions (1 mM) and further dilutions were prepared with deionized water. Nanopure deionized water (specific resistance >18.2 M Ω cm) used throughout this work was supplied by a Barnstead water purification system model D8961. All chemicals were reagent grade and were used as received.

Melanin Synthesis: Melanin particles were synthesized using procedures modified from previous work in our lab (Bayachou et al. ECS Trans. 80(10) (2017) 1447-1458). A DAI solution (125 mmoles) in 12.5 mL ethanol was hydrolyzed by mixing with 2.5 mL aqueous NaOH solution (2-mole equivalent, i.e. 250 mmoles). This reaction mixture was diluted with DI water to a final volume of 50 mL. The solution rapidly turned dark violet and became black upon further stirring. During a ten-minute reaction time, nanoparticles of black melanin in the form of a precipitate were obtained. After decanting much of the aqueous supernatant, the wet melanin particles were dried under gentle heat until all remaining water evaporated. The resulting solid material was then air-dried at room temperature overnight. The material from this process is labeled as LSM (low sodium melanin). Another slightly modified synthesis process where the mole equivalent of sodium hydroxide is increased by 20% resulted in a material with a slightly different behavior and is labeled HSM (for high sodium melanin). For this material, the hydrolysis of 125 mmole of DAI was conducted in the presence of 300 mmoles of NaOH.

Metal adsorption: Metal ion solutions (50 ppm, 15 mL volumes) were used for metal adsorptions. LSM and HSM samples (7 mg) were used for extraction. The samples were mixed, and intermittent shaking was maintained for 30 minutes, unless otherwise indicated. The supernatant was isolated from the particles by syringe fitted with appropriate filter (VWR 25mm filter, 0.2 μm polypropylene membrane).

Instrumentation and Sample Preparation

Scanning Electron Microscopy (SEM, Hitachi S-4500) was used to compare the general morphology and melanin particle size, before and after metal extraction. The dried melanin samples were gently pressed onto carbon tape for imaging and elemental analysis by *Energy Dispersive X-Ray Spectroscopy* (EDS).

Internal-Reflection, Fourier-Transform Infrared Spectroscopy (FTIR, Perkin Elmer Spectrum Two with UATR accessory) was performed in the range of 4000–450 cm^{-1} , with a 4 cm^{-1} peak resolution. Dried melanin samples, before and after metal extraction, were compressed directly onto the diamond single-reflection ATR prism, with signal averaging over 4 scans.

X-Ray Photoelectron Spectroscopy (XPS, PHI 5000 Versa Probe, Al mono X-ray, 50 W) with depth profiling was used for qualitative assessment of zinc concentrations in melanin after metal extraction. The dried melanin was mounted onto carbon-tape for analysis. A survey scan (10 min) compared elemental content before and after a 3-min Ar ion-beam sputter for depth profiling.

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES, Perkin Elmer Optima 7000 DV) used an argon-based plasma to quantify the metal concentrations in solution; calibrations for each metal ion were determined over concentrations ranges of 1 to 15 ppm and 10 to 50 ppm. Supernatant solutions after metal extraction were used without further dilution. For analysis of the metal content of melanin particles after metal extraction, the particles were dissolved completely by adding concentrated nitric acid (1 mL) and hydrogen peroxide (30%, 1 mL) then heated to approximately 100 °C until complete degradation of melanin particles leaving behind water soluble metal nitrates that were dissolved in 5 ml of deionized water.

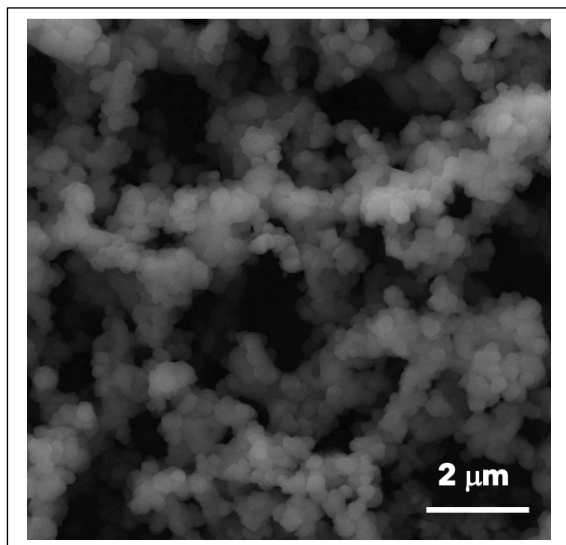


Figure S1. SEM image of electropolymerized melanin film from electrochemical oxidation of DHI in ethanol-50 mM phosphate buffer solution.

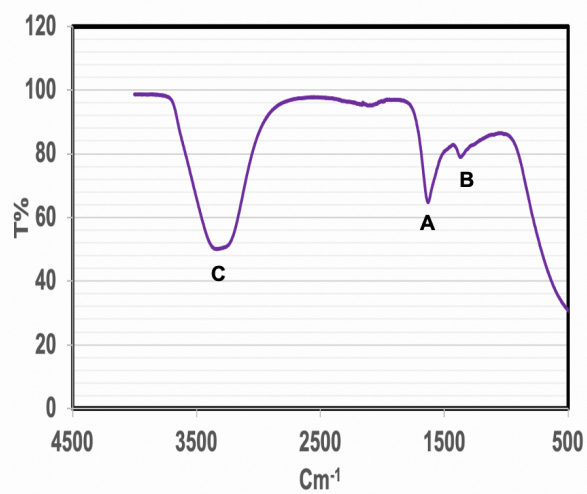


Figure S2. Fourier Transform IR analysis of synthetic melanin prepared in this work. The IR spectrum shows characteristic peaks reported for natural melanin. Feature A is characteristic of carboxylate groups and nitrogen-containing heterocycles. The *o*-hydroxy quinone groups are featured in peak B. Feature C represents the catechol groups of melanin.

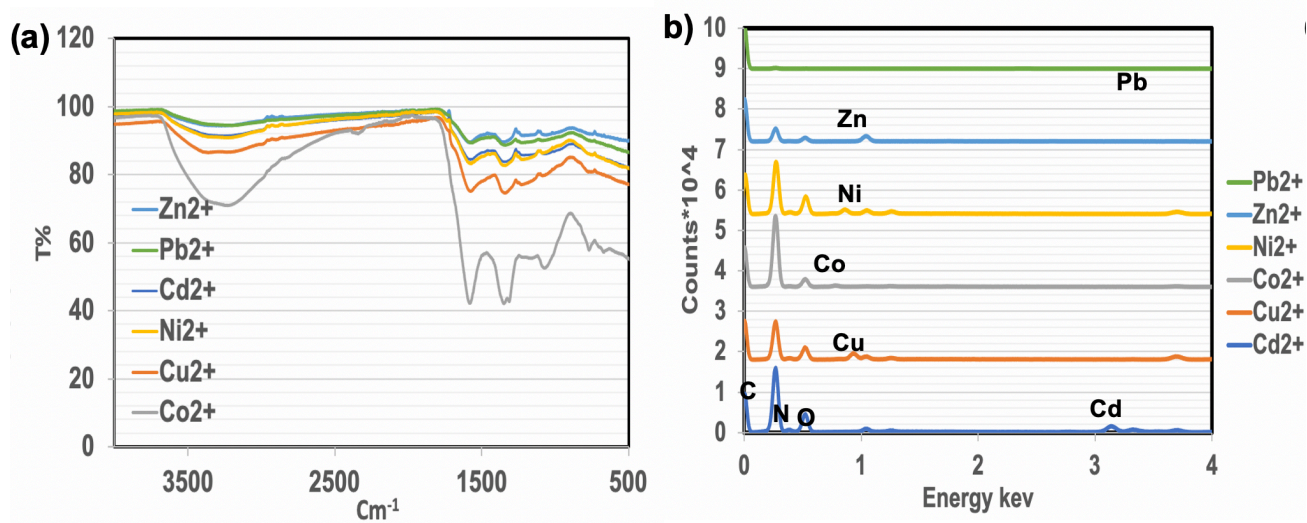


Figure S3. (a) Fourier Transform IR spectra of synthetic melanin after extraction of the various metal divalent cations from aqueous solution. Table S1 in supporting information shows shifts of the melanin peaks after adsorption of metal ions. (b) Energy Dispersive X-ray Spectroscopy confirmed the incorporation of metal divalent cations in the melanin nanoparticles.

Table S1. IR Peak wavenumbers for LSM Synthetic Melanin before and after metal ion adsorption

	A (cm ⁻¹)	B (cm ⁻¹)
melanin	1581	1354
Cd ²⁺	1580	1346
Cu ²⁺	1578	1340
Co ^{2+a}	1581	1349 1313
Ni ²⁺	1574	1339
Zn ²⁺	1580	1346
Pb ²⁺	1577	1340

^aNote the split peak B after Co²⁺ adsorption on the synthetic melanin sample.

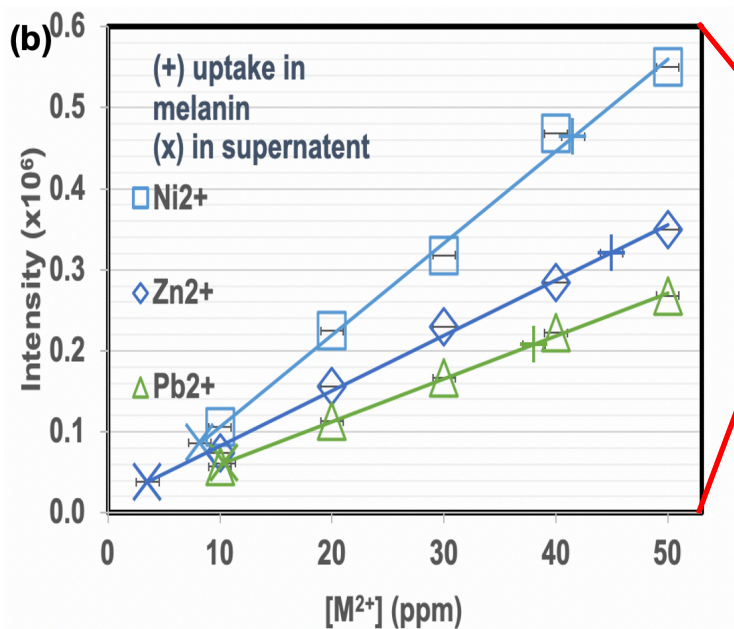
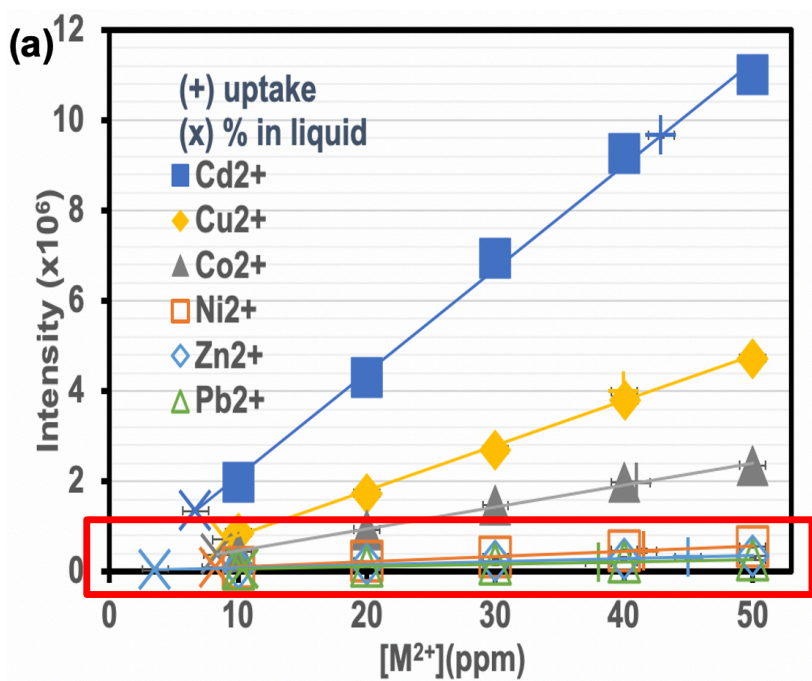


Figure S4. (a) Linear calibration data used in the quantification of metal ion uptake by melanin particles melanin (7 mg melanin and 50 ppm starting metal ion concentration). A few measurement points for both uptake (+) and are quantity left in supernatant (x) are also shown on the calibration curves. (b) The lower intensities are zoomed out for clarity.

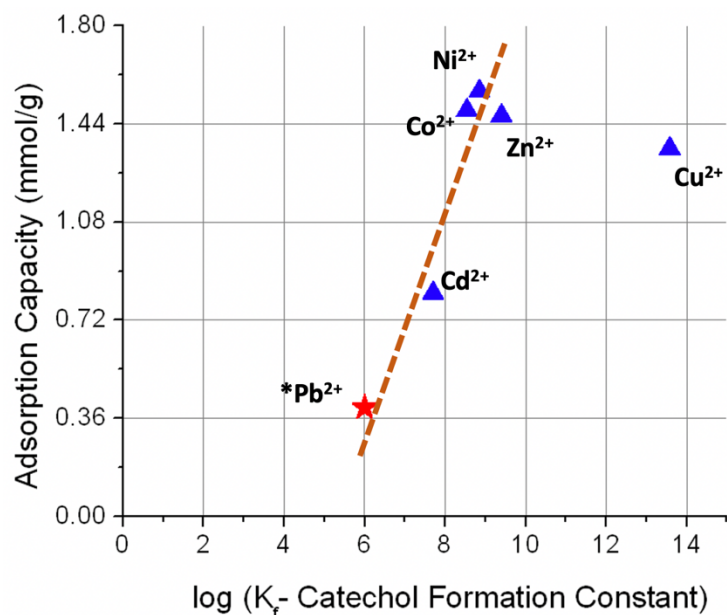


Figure S5. Adsorption capacities as a function of formation constant of catechol-metal ion complex. The dashed line is a linear trend showing the break after the data of Ni²⁺. *The formation constant of the lead complex represented by the star on the graph is only an estimate based on the trend.