Supplementary Information

Chiral Resolution of Copper Aspartate under Reaction-Diffusion: Synergy of Experiment and Simulation

Poh Ying Fong,[a] Sehrish Khan,[a] Hatem M. Titi,[b] Manal Ammar,[c] Fiorenzo Vetrone,[d] Mazen Al-Ghoul [c]* and Louis A. Cuccia [a]*

[a] Department of Chemistry and Biochemistry, Concordia University, 7141 Sherbrooke Street West, H4B 1R6 Montreal, Canada

[b] Department of Chemistry, McGill University, 801 Sherbrooke Street West, H3A 0B8 Montreal, Canada

[c] Department of Chemistry, American University of Beirut, P.O. Box 11-0236, Riad El-Solh, 1107 2020 Beirut, Lebanon

[d] Centre Énergie, Matériaux et Télécommunications, Institut National de la Recherche Scientifique, Université du Québec, 1650 Boul. Lionel-Boulet, J3X 1P7 Varennes, Canada

Materials and methods

Materials

All chemical reagents, L-aspartic acid (from Aldrich Chemical Company), D-aspartic acid (from Alfa Aesar), DL-aspartic acid, L-proline, D-proline (from TCI America), BD™ Difco™ Agar (from Fisher Scientific; Cat# 281230), sodium hydroxide (from BDH Reagents and Chemicals), and copper nitrate trihydrate (from Thermo Scientific Chemicals), were used without further purification. All electrolyte solutions and hydrogels were freshly prepared using Millipore water (18.2 mΩ). Glass test tubes (20 mm × 125 mm) were used.

Coordination polymerization of homochiral and racemic CuAsp in agar gel

CuAsp coordination polymers were synthesized in test tubes containing both inner and outer electrolytes. Initially, the inner gel solution was prepared by dissolving 0.6 g (1% wt/wt) agar powder in 54 mL of water, heating (80 - 85 ºC), and stirring until completely dissolved. The agar gel was then cooled to 60 ºC. Concurrently, a solution of deprotonated L-, D-, or DL-Asp was prepared by dissolving 0.3993 g (3 mmol) of Asp and 0.24 g (6 mmol) of NaOH in water (6 mL). This solution was then added to the cooled agar gel and stirred for 5 minutes. The mixture was carefully transferred to test tubes, covered, and left to solidify fully (~4 hours). The final concentration of the inner electrolyte was 50 mM Asp and 100 mM NaOH, filling each tube with a total gel volume of 18 mL. The outer electrolyte, composed of copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) 1.4496 g (6 mmol, 300 mM), was dissolved in water (20 mL) and carefully layered over the gel using a glass Pasteur pipette. The test tubes were sealed with parafilm and left undisturbed to facilitate the controlled diffusion of electrolytes and the formation of distinctive blue spherulitic crystals.

The CuAsp spherulites were collected after the diffusion process was completed. The agar gel was carefully extracted and segmented into different zones using a spatula. Each zone underwent 3-5 washes in hot water to effectively separate the spherulites from the agar gel matrix. The spherulites were transferred to Corning® 15 mL centrifuge tubes, followed by centrifugation at 450 xg for 5 minutes, using an IEC clinical CL bench-model centrifuge equipped with a Rotor 809 (radius = 12.7 cm). Finally, the spherulites were dried overnight in a 60 ºC oven.

Chiral separation of DL-Asp using homochiral Pro-copper complexes in agar gel

The inner electrolyte, consisting of 1 %wt/wt agar and deprotonated DL-Asp, was prepared as described above. The outer electrolyte was then prepared by dissolving 0.2303 g (2 mmol, 100 mM), and 0.4605 g (4 mmol, 200 mM) of L- or D-Pro in water (20 mL), followed by the addition of 0.08 g (2 mmol, 100 mM), and 0.16 g (4 mmol, 200 mM) of NaOH for deprotonation. Subsequently, 1.4496 g (6 mmol, 300 mM) of $Cu(NO_3)_2 \cdot 3H_2O$ was added to the deprotonated Pro solution with continuous stirring for 5 minutes until fully dissolved. This outer electrolyte was gently layered over the gel using a glass Pasteur pipette. The test tubes were sealed with Parafilm and left undisturbed. After two weeks, the gels were extracted, and the spherulites were isolated as outlined above.

Solid-state circular dichroism (Solid-state CD)

CD spectra were recorded in the range of 400 to 800 nm using a Jasco J-710 spectropolarimeter. The spectra were obtained with a resolution of 5 points/nm, and each curve represents the accumulation of 5 scans at a scan rate of 100 nm/min. A binomial filter with a convolution width of 99 points was applied. Solid-state samples were prepared as Nujol mulls by grinding the samples (2.0 - 2.8 mg) from each zone in a mortar and pestle in Nujol mineral oil to achieve a homogeneous mixture (30 - 35 %wt/wt). This mixture was

then sandwiched between two quartz discs (22.5 mm diameter, 1 mm thick), and the sample assembly was mounted in the instrument using a circular cell holder.

Powder X-ray diffraction (PXRD)
PXRD patterns were collected on a Rigaku MiniFlex 6G X-ray diffractometer equipped with Cu*K*α radiation (λ = 1.54059 Å) and a sealed-tube X-ray source operating at 40 kV and 15 mA. Samples were smeared onto a zero-diffraction silicon wafer and measured between the 2θ of 3 - 50 in 0.01º step with a scan rate of 10 º/min.

Scanning electron microscopy imaging (SEM)
SEM images were acquired using a Tescan Mira instrument with an accelerating voltage of 15 kV. Prior to imaging, SEM samples were coated with a thin layer of platinum.

Nitrogen adsorption-desorption isotherms
The N_2 adsorption-desorption isotherms were measured at 77 K on a 3Flex Micromeritics instrument. Prior to the measurement, the sample was washed with DMF and DCM, and subsequently dried in a vacuum oven at 70 ºC overnight.

Attenuated total reflectance Fourier-transform infrared (ATR-FTIR)
ATR-FTIR spectra of samples in the form of powders were collected using a Bruker Alpha II FT-IR spectrometer. All spectra were recorded in the wavelength range from 400 cm^{-1} to 4000 cm^{-1}.

Variable temperature-Powder X-ray diffraction (VT-PXRD)
The thermal events for L-CuAsp were performed on a Bruker D8 Advance instrument equipped with a LYNXEYE detector using nickel-filtered Cu*K*α radiation. The setup was equipped with an Anton Paar CHC^+ chamber. Diffractograms were collected stepwise in a dry environment. After each pattern was recorded for 6 min, the chamber temperature was raised by 5 °C at a heating rate of 30 ºC/min and kept for isotherm at the destination temperature for 1 min before the new scan was collected.

Thermogravimetric analysis (TGA)
TGA and differential scanning calorimetry (DSC) data were measured on a TGA/DSC 1 (Mettler-Toledo, Columbus, Ohio, USA) instrument. Samples (5-6 mg) were placed in alumina crucibles. All measurements were carried under a 25 mL/min stream of air, and the samples were heated from room temperature up to 600 ºC using a constant heating ramp of 10 ºC/min.

Dynamic vapour sorption (DVS)
Prior to the experiment the sample was dried at 100 ºC, for four hours. The experiment was conducted on a surface measurement system (DVS) using a full cycle sorption desorption method. The sorption cycle starts from 0 RH% to 95 RH% sorption with an increment of 5 RH% followed by desorption to 0 RH%, in which the temperature was kept constant at 25 ºC during the measurement on a L-CuAsp sample of 1.6633 mg. At each RH% step, the material will be allowed to equilibrate dm/dt of 0.002% for a period between 10 - 360 min.

UV-Vis spectrophotometry
UV-Vis absorbance spectra were recorded in the range of 200 to 900 nm using a Varian Cary 100 Bio UV-Vis spectrophotometer. The following sample concentrations were prepared: (1) L- and D-Pro solutions at 10 mg/mL (20 mg of Pro in 2 mL of Millipore water), (2) a 10-fold dilution of 300 mM $Cu(NO_3)_2 \cdot 3H_2O$, 100 mM L- and D-Pro-copper complexes, and (3) a 20-fold dilution for the 200 mM L- and D-Pro-copper complexes. Each solution sample was loaded in a quartz cuvette with an optical path of 2 mm for analysis.

Coordination polymerization and characterization of L-, D-, and DL-CuAsp in agar gel

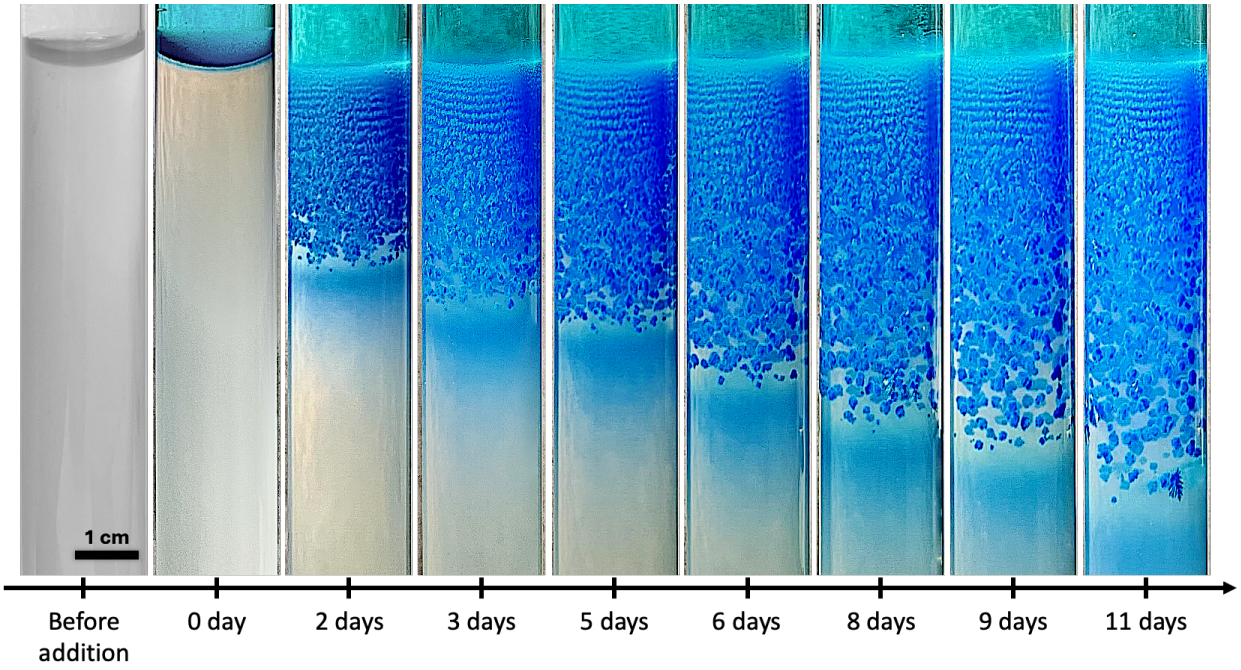

Figure S1. Time-lapse images of D-CuAsp periodic precipitation.

Attenuated total reflectance Fourier-Transform infrared (ATR-FTIR)

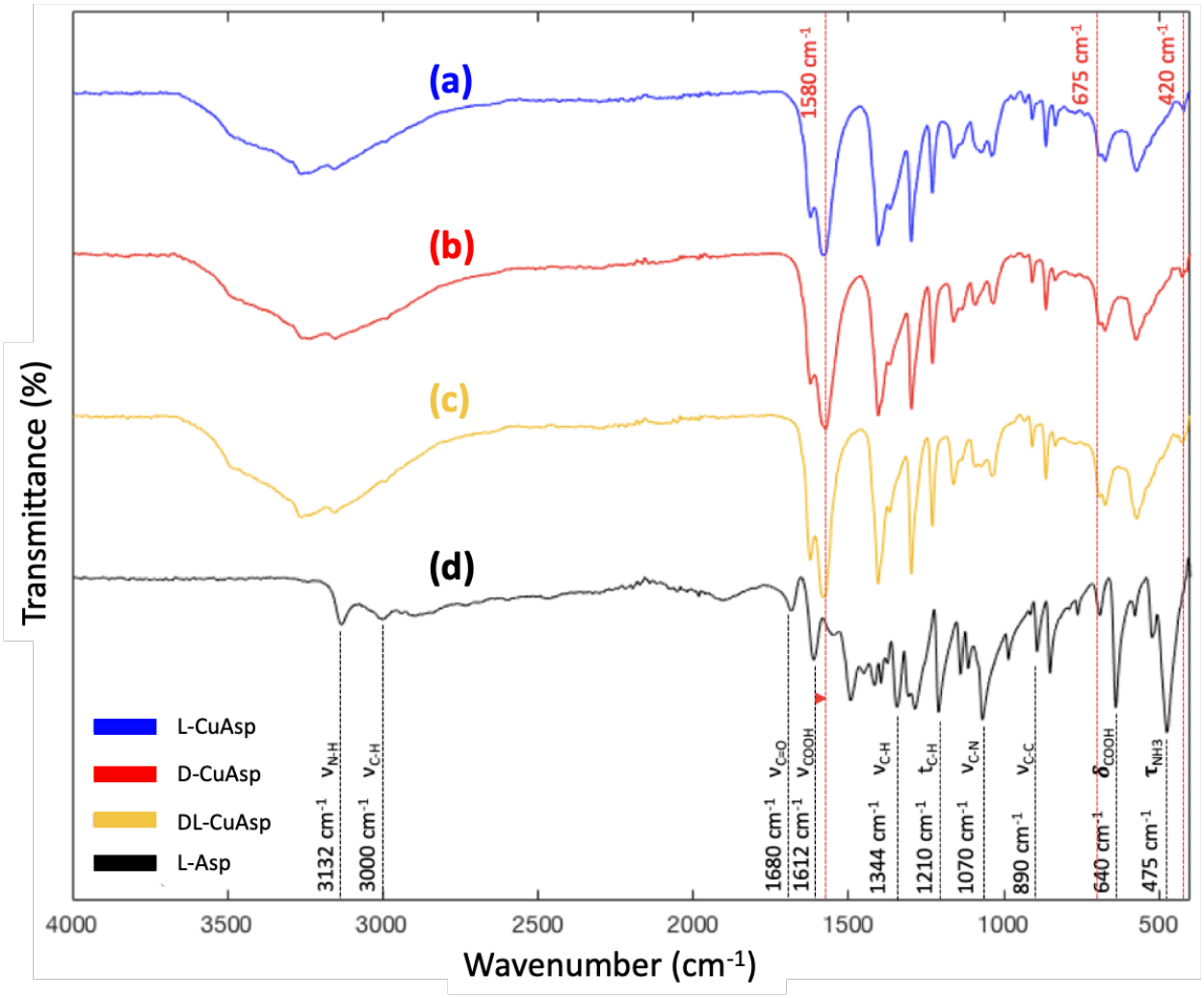

Figure S2. ATR-FTIR transmittance spectra of (a) L-, (b) D-, and (c) DL-CuAsp compared with (d) L-aspartic acid.

Nitrogen isotherm and Brunauer-Emmett-Teller (BET) analysis

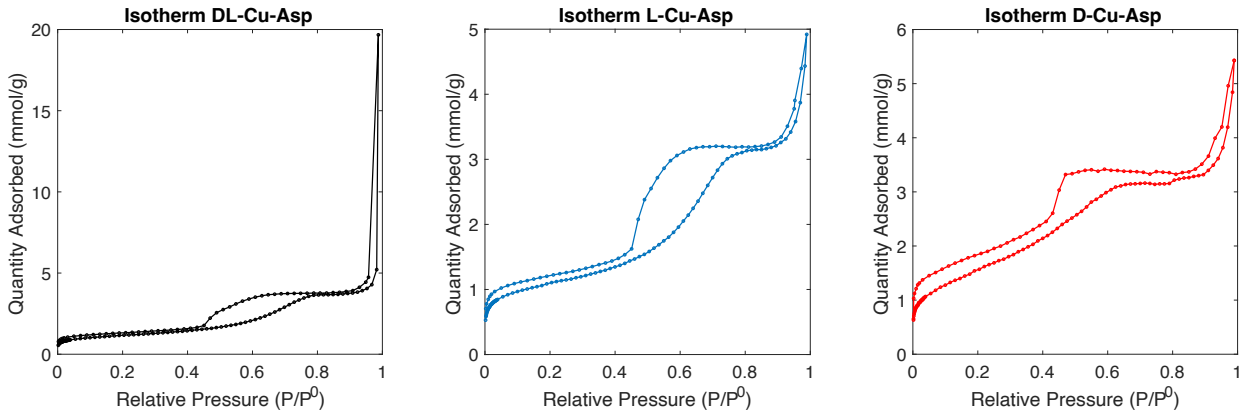

Figure S3. Nitrogen adsorption–desorption isotherms at 77 K for Cu-aspartate coordination polymers: (a) DL-CuAsp, (b) L-CuAsp, and (c) D-CuAsp. All exhibit Type IV isotherms with H3-type hysteresis loops, indicative of mesoporosity. Despite having identical PXRD patterns, DL-CuAsp shows significantly larger total pore volume (0.684 cm^3/g) and average pore diameter (~294 Å), reflecting looser packing or aggregated morphology. L-CuAsp and D-CuAsp display similar isotherms and BET areas (L-CuAsp: 88.1 m^2/g, D-CuAsp: 93.7 m^2/g), consistent with their mirror-image crystal structures. Minor differences in micropore values are attributed to measurement variation rather than structural disparity.

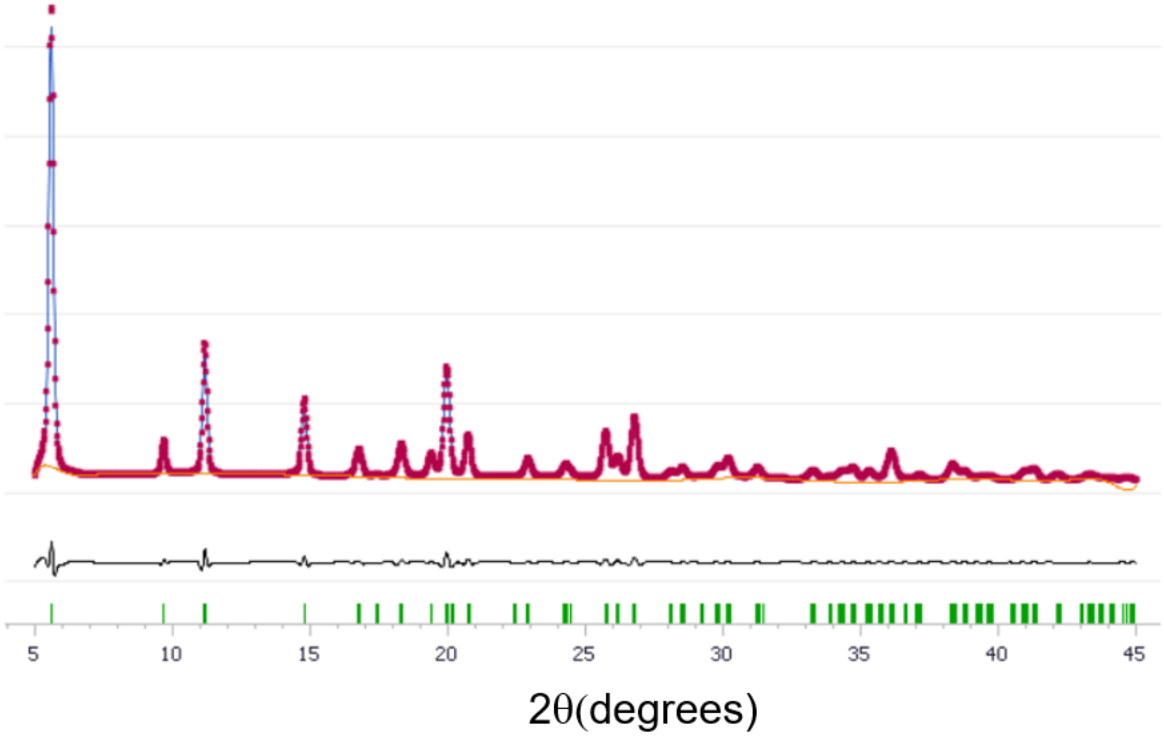

Figure S4. Pawley refinement plot of the L-copper aspartate (L-CuAsp) coordination polymer based on powder X-ray diffraction (PXRD) data. The experimental pattern (red dots) shows excellent agreement with the fitted profile (blue line), and the difference curve (black) exhibits minimal residuals, confirming the accuracy of the unit cell and peak modeling. Bragg reflection positions (green ticks) correspond to the expected peaks for the monoclinic phase with space group *P* 1 2 1. The refinement, performed over a 2θ range of 5–45° using Cu Kα radiation (λ_1 = 1.5406 Å), produced low residual factors: Rwp = 3.87%, Rp = 2.87%, and Rwp (without background) = 7.47%. Fixed lattice parameters were a = 22.07 Å, b = 9.17 Å, c = 5.10 Å, and β = 133.95°, consistent with the target phase. The pseudo-Voigt profile function and Bragg-Brentano geometry were employed. The refined crystallite size parameter (C = 13.56 ± 0.28 nm) suggests nanocrystalline domain dimensions. Although no structural model was refined, the quality of the Pawley fit strongly supports phase purity and high crystallinity of the synthesized L-CuAsp coordination polymer.

Solid-state circular dichroism (CD) spectra of DL-CuAsp individual spherulites

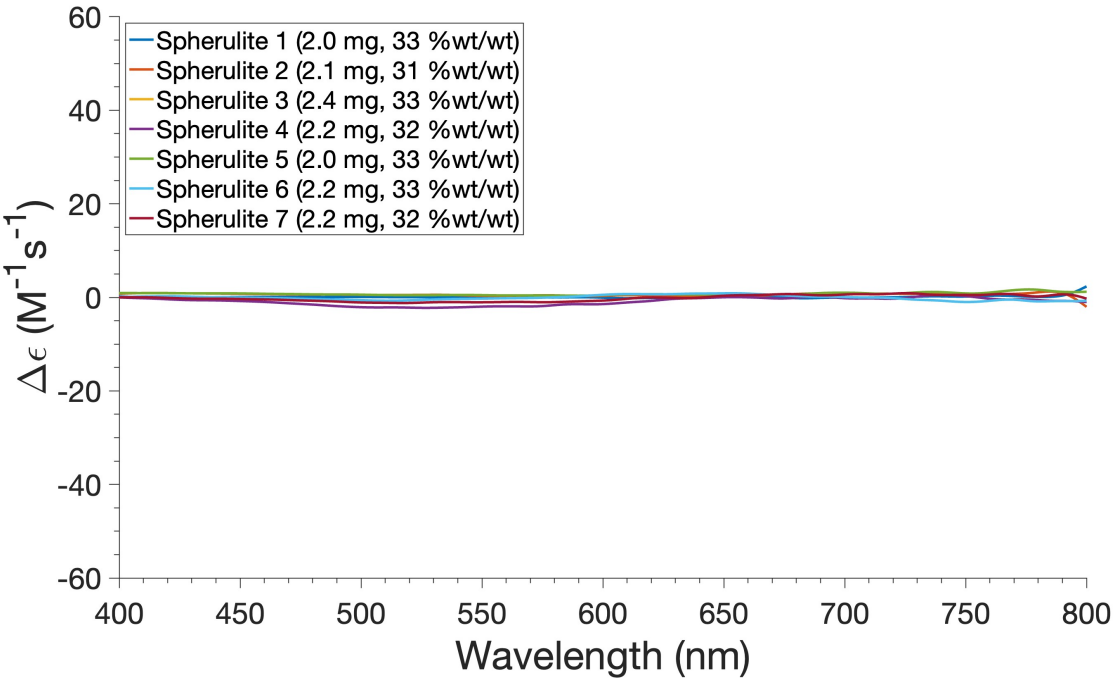

Figure S5. Solid-state CD for individual spherulites of DL-CuAsp.

UV-Vis spectra

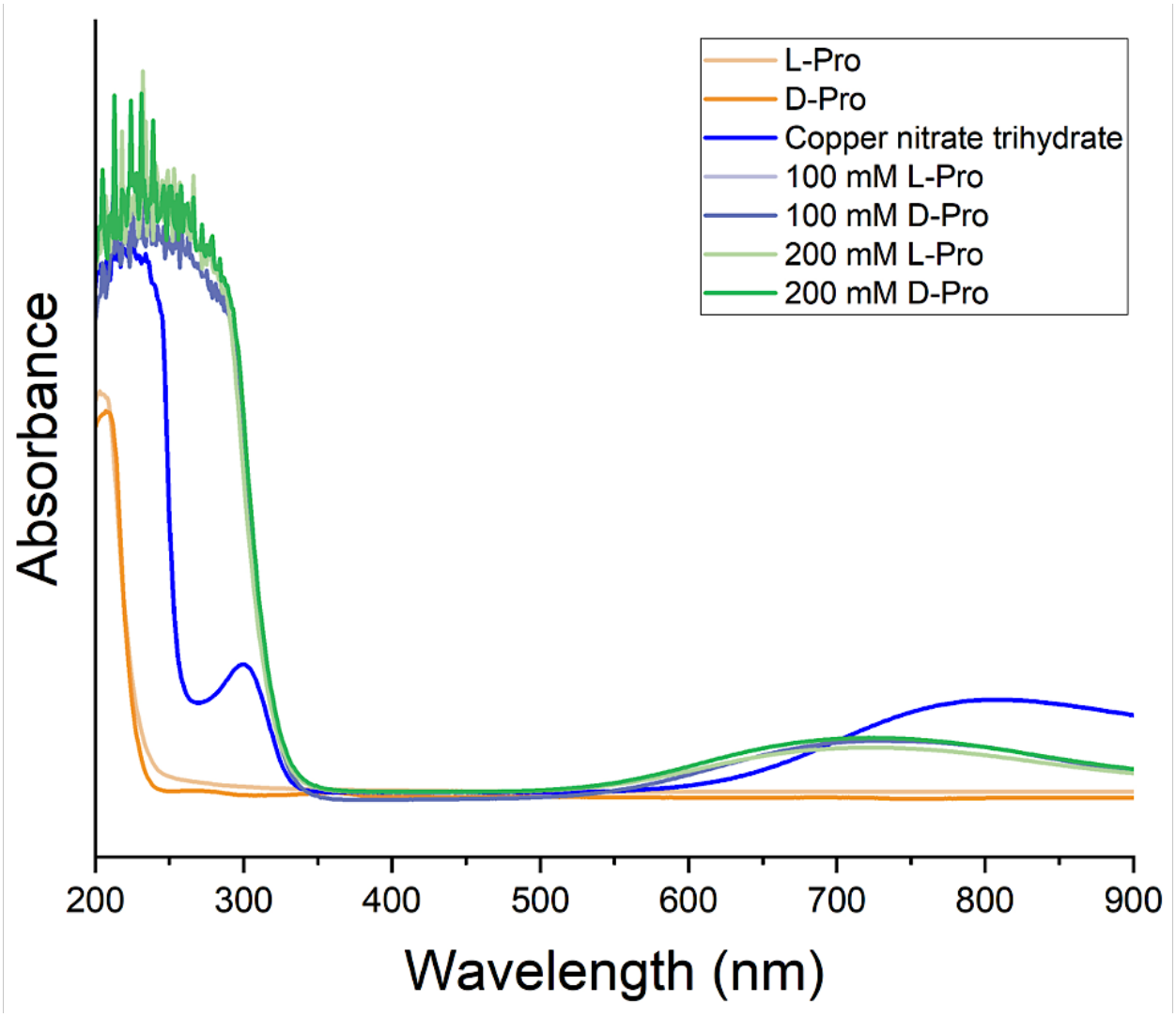

Figure S6. UV-Vis spectra of the starting materials and copper complexes with 100 mM and 200 mM L- and D-Pro.

Chiral resolution of DL-Asp in the presence of homochiral Pro-copper complexes in agar gel

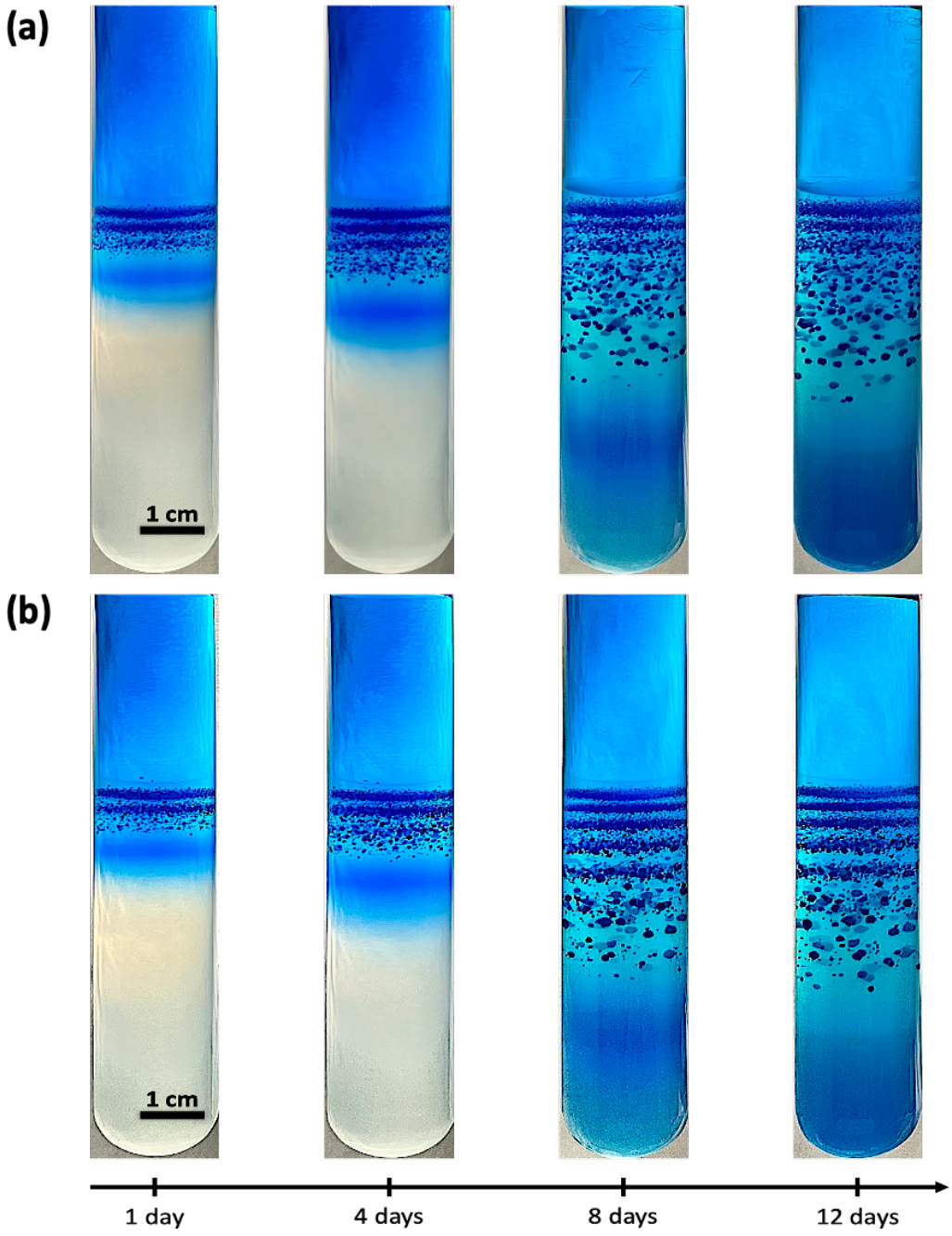

Figure S7. Periodic precipitation of chiral resolution of DL-Asp in the presence of L- and D-Pro-copper complex.