SOP 4: Cu(II) Chelation using D-glucosamine and UV-Vis Assay

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1. Purpose / Scope

- Uses D-glucosamine as the substitute for our MAC
  - MAC cannot be ordered as is, and production amounts are uncertain and not guaranteed
  - D-glucosamine was originally the positive control
- Uses Cu(II) as substitute for cisplatin
  - Cisplatin is a cytotoxic drug and unsafe for us to use in the lab
  - Cu(II) provides a safe and inexpensive alternative
- Measurement uses UV-Vis to confirm whether the ligand has complexed with Cu(II)
- Instead of committing to one pH, the tubes are walked up from pH 5, pH 6, pH 7 and pH 8 – removes the risk of choosing a pH that is less optimal and gives a trend. However, this would mean 4 scans per tube instead of one – If we are very short on time, picking fewer pH levels would be better.
- (optional) If the shift is clear by pH 6 (or pH 7), stop early and use that as your working pH

2. Reaction: Ligand → Product

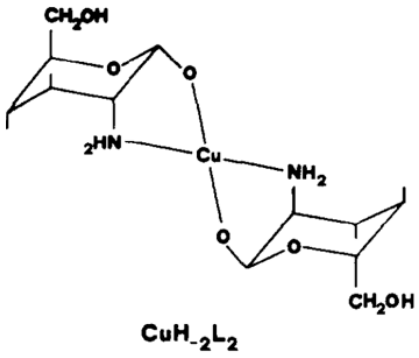
Reactant (ligand)	Process	Product(s)
D-glucosamine	Complexation of Cu(II)	Cu(II)–D-glucosamine complex.

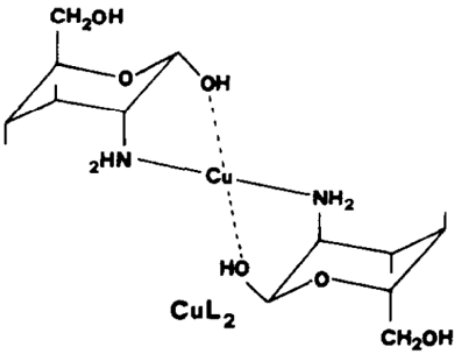
Complexation

1. **Copper(II) complexation by D-glucosamine. Spectroscopic and potentiometric studies;** https://doi.org/10.1016/S002-0-1693(00)80688-X

Notes:

- Confirming the 1:2, metal:ligand structure and comparing with a negative control (acetylated glucosamine – expected not to bind to copper).
- 2 Prominent structures (see below):





- $CuH_{-2}L_2$
  - 90% at pH 8.1
  - Deprotonated hydroxyls
- CuL_2
  - 55% at pH 6.9

3. Materials & Reagents

Reagent	Amount	Makes
CuSO4·5H2O (MW 249.7)	0.624 g	25 mL of 100 mM copper stock
D-glucosamine hydrochloride (MW 179.2)	1.078 g	25 mL of 200 mM ligand stock
N-acetyl-D-glucosamine (MW 221.2)	1.106 g	25 mL of 200 mM control stock
NaCl	0.877 g per 100 mL	0.15 M background salt – use it as the solvent for everything
NaOH (0.1 M)	Small volume additions	pH adjustment, dropwise
HCl (0.1 M)	Small volume additions	pH adjustment, dropwise

Equipment

- UV-Vis Spectrophotometer (400 – 900 nm range)
- Balance
- pH meter
- Calibrated electrode
- 1 cm cuvettes
- 25 mL volumetric flasks (x4)
- 10 mL tubes or small volumetric flasks (x3 – if not making fresh tubes for each pH)
- Micropipettes and pipette tips
- Fine-tip dropper or small syringe for acid/base addition
- Vortex mixer

4. Reaction Conditions

Parameter	Value / Details (fill in)
pH	*5.0, 6.0, 7.0 and 8.0*
Temperature	*25 °C*
Metal concentration	*10 mM*
Ligand concentration	*20 mM*
Sample volume	*10 mL*
Sample time	*2 minutes after reaching target pH*
Detection / readout method	*UV-Vis*

5. Step-by-Step Procedure

Tube	Copper stock	Ligand stock	Make up to 10 mL with 0.15 M NaCl
1 Copper only	1.0 mL	—	→ 10 mM Cu
2 Test	1.0 mL	1.0 mL glucosamine	→ 10 mM Cu, 20 mM ligand (1:2)
3 Negative control	1.0 mL	1.0 mL N-Acetyl-D-glucosamine (GlcNAc)	→ 10 mM Cu, 20 mM ligand (1:2)

1. Make the three stocks in 0.15 M NaCl (copper stock, ligand stock and control stock)
2. Make up tubes 1, 2 and 3 as in the table above.
3. Bring all three tubes to pH 5.0 with 0.1 M HCl or NaOH, dropwise, checking with a meter.
4. Zero the spectrophotometer on 0.15 M NaCl. Scan each tube from 400 to 900 nm in a 1 cm cell. Record peak wavelength and absorbance, and note any cloudiness.
5. Take all three tubes up to pH 6.0 and scan again. Then pH 7.0 and scan. Then pH 8.0 and scan.
6. Centrifuge and read the supernatant for any tube that has gone cloudy, recording that you did so.
7. Plot peak wavelength against pH for all three tubes on one chart.

6. Controls

Control	Composition / Expected Result
Positive Control	N/A – the glucosamine is already a positive control
Negative Control	N-Acetyl-D-glucosamine (GlcNAc) – shows little to no movement as pH changes (similar peak), but cloudiness should increase at higher pH.

7. Notes, Safety, and Troubleshooting

Notes

- The result is the shape of the trend, not a single reading:

- Tube 2's peak should move steadily to shorter wavelength as pH rises, and grows taller. Published anchors: near 660 nm around pH 7 (CuL2) and near 620 nm around pH 8 (CuH-2L2).
- Tube 1 and tube 3 should show little or no movement. Tube 3 tracking tube 1 across the whole series is a stronger negative control than a single point.
- Expect little to nothing at pH 5 in any tube as the amine is still protonated there. A flat pH 5 point is information, not a failed run.
- If the shift is clear by pH 6, you can stop early and use that as your working pH from then on.
- Check the pH with a meter AFTER mixing, not before. Copper binding releases protons and pulls the pH down on its own, so a tube you set correctly beforehand will drift.
- Do not leave the tubes standing. Tamura 1965 reports glucosamine being decomposed by copper in alkaline solution unless handled quickly. Mix, adjust, scan.
- Use the same cell for every scan, and zero on 0.15 M NaCl with no copper in it, never on tube 1.
- Adding acid and base dilutes the tubes as you go. Keep the added volumes small and record them. If you add more than a few per cent of the tube volume, absorbances across the series are no longer strictly comparable.

Safety

Substance	Hazard	Precaution
$CuSO_4 \cdot 5H_2O$	Irritant (skin, eyes, respiratory if dust inhaled) and harmful if swallowed	Gloves, eye protection when weighing; avoid generating dust
D-glucosamine·HCl	Low hazard	Standard handling
N-acetyl-D-glucosamine	Low hazard	Standard handling
NaCl	Low hazard	Standard handling
NaOH (0.1 M)	Irritant/corrosive to skin and eyes, even dilute	Gloves, eye protection; add dropwise, don't splash
HCl (0.1 M)	Irritant/corrosive to skin and eyes, even dilute	Gloves, eye protection; add dropwise, don't splash
Copper-containing waste (all three tubes, every pH point)	Heavy-metal waste — not drain-disposable	Collect in a labelled aqueous heavy-metal waste container

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Troubleshooting

Symptom	Cause	Action
Tube 3 going cloudy due to copper hydroxide formation	Micera 1985 reports copper hydroxide precipitating above pH 5 when nothing binds. This is the expected negative result, not an indication of a mistake	Spin it down and read the supernatant, but do not calculate molar absorbance.
Little to no change at pH 5	Amine is often still protonated at pH 5	This is not a failed run – a flat point at pH 5 is information

Symptom	Cause	Action
No shift in tube 2 even at pH 8	Ligand excess too low, stock decomposed, or pH wasn't actually re-checked after mixing	Confirm 1:2 ratio held, use freshly made glucosamine stock, always check pH after mixing not before
Tube 2 also goes cloudy like tube 3	Not enough ligand, or copper stock concentration off	Recheck stock concentrations or consider a higher ligand excess
Changes seen in the negative control	The tube was left to sit for too long or has been contaminated	Discard and remake the tube
pH keeps drifting and won't settle	Electrode needs recalibration, or you're reading too soon after an addition (binding itself releases protons, which takes a moment to settle)	Recalibrate before starting; wait for the reading to stabilise before recording, but don't let the tube sit long past that
Scans across the series don't line up cleanly	Instrument zeroed on the wrong blank, or dilution from repeated acid/base additions has crept up	Always zero on 0.15 M NaCl (never tube 1); keep a running total of added acid/base volume and flag if it exceeds a few % of tube volume

8. Literature Source

Citation 1	Details
Author(s)	G. Micera, S. Deiana, A. Dessi', P. Decock, B. Dubois, H. Kozłowski
Title	Copper(II) complexation by D-glucosamine. Spectroscopic and potentiometric studies
Year	1985
DOI / URL	https://doi.org/10.1016/S0020-1693(00)80688-X
Page / Section referenced	

Citation 2	Details
Author(s)	J. Lerivrey, B. Dubois, P. Decock, G. Micera, J. Urbanska, H. Kozłowski
Title	Formation of D-glucosamine complexes with Cu(II), Ni(II) and Co(II) ions
Year	1986
DOI / URL	https://doi.org/10.1016/S0020-1693(00)81209-8
Page / Section referenced	

Citation 3	Details
Author(s)	Zenzo Tamura, Motoichi Miyazaki
Title	Metal Complexes of D-Glucosamine and its Derivatives. V. Spectrophotometric Investigations on Copper (II), Nickel (II), and Cobalt (II) Complexes of D-Glucosamine and the Preparation of Glucosamine-copper Complex
Year	1965
DOI / URL	https://doi.org/10.1248/cpb.13.345
Page / Section referenced	

Numbers taken from the papers:

- 660 nm, ε = 44 M-1 cm-1 for CuL2 and 620 nm, ε = 40 for CuH-2L2 — Micera et al. 1985, Inorg. Chim. Acta 107, 45–48.
- CuL2 is ~55% of the copper at pH 6.9; CuH-2L2 is ~90% at pH 8.1; amine pKa 7.70 — same paper.
- N-acetyl-D-glucosamine shows no complex formation and no shift of the d–d maximum from the free-ion position; copper hydroxide precipitates above pH 5 — same paper.
- Maximum moves from about 800 nm towards 600 nm as pH rises, and absorbance rises between 500 and 350 nm; no precipitation seen at any pH in a 2:1 ligand:metal series — Tamura & Miyazaki 1965, Chem. Pharm. Bull. 13, 345–351.

Concentrations, volumes and the 0.15 M NaCl background are practical choices, not values from the papers. The papers give no free-copper absorbance, so tube 1 is your own reference.