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WG1 - NECTAR for highly hydrolysable (HHC) and/or low-valence state (LVC) cations has prepared the recommendations for working with copper(I) ions in aqueous solution. This work is related to the task of WG1 defining the most appropriate and accurate procedures and experimental approaches for the study of the solution speciation of LVC (e.g., Fe(II), Sn(II), Cu(I)).

The NECTAR recommendations for solution studies with copper(I) ions in aqueous media

All reagents should be of the highest purity and all laboratory vessels should be acid washed in 1 M HCl (or HNO_3) and subsequently rinsed with 18.2 MΩ·cm (or 0.1 µS.cm^{-1}) Milli-Q water to minimize trace metal contamination. When preparing buffers, it is recommended to pass the buffer stock solutions through a Chelex-100 ion exchange resin to remove trace metal impurities.

Working with Cu(I) in aqueous media is challenging due to its inherent instability to oxidation and disproportionation, therefore, it is strongly recomended to do all the experiments under anaerobic conditions (if possible in a glovebox and with a concentration of oxygen preferably below 0.5 ppm) and using deoxygenated solvents. Deoxygenated water can be prepared by boiling Milli-Q water for 1-2 h and allowing to cool under a nitrogen or argon atmosphere. Alternatevely, the boiling of water can be carried out under lower pressure at laboratory temperature. It is advised to prepare the solvent one day in advance and store the solution in a glovebox. Organic solvents, such as acetonitrile (used to prepare Cu(I) stock solutions, see below), can be deoxygenated using Schlenk vessels. Three to four iterations of freeze-pump-thaw cycles or rigorous vacuum degassing followed by purging with nitrogen or argon, and keeping the solution in the glovebox are recommended (check the compatibility of your glovebox to the organic solvents). Alternatively, in the absence of a glovebox, you can keep your water and organic solvents under inert gas (nitrogen or argon) in Schlenk vessels, and purge daily with a nitrogen (or argon)/vacuum line to guaranty the absence of oxygen. The

concentration of oxygen dissolved in solvents can be estimated by the analytical methods discussed below.

- **The control of oxygen level in water**

The concentration of oxygen dissolved in aquoues solution can be determined by several analytical procedures. Instrumental analysis is based on voltametric analysis,[1] measurement of limiting electric current at potentials lower than -0.45 and -1.35 V (vs. NHE) for two waves representing two-electron oxygen reduction:

$$O_2 + 2\ H^+ + 2\ e^- \Leftrightarrow H_2O_2$$
$$H_2O_2 + 2\ H^+ + 2\ e^- \Leftrightarrow 2\ H_2O$$

Another approach is based on the measurement of the luminescence-decay quenching effect of oxygen on Ru(II) complexes of phenonathroline derivatives which are placed on top of optode.[2] Both instrumentations are commercially available, but they require careful calibration of the instrument based on the knowledge of the oxygen concentration by an analytical independent method.

Another possible approach is the redox titration procedure proposed by Winkler:[3]

$$2\ Mn^{2+}\ (aq) + O_2\ (aq) + 4OH^- \rightarrow 2\ MnO(OH)_2(s)$$
$$MnO(OH)_2(s) + 4\ H^+(aq) \rightarrow Mn^{4+}(aq) + 3\ H_2O(l)$$

Freshly precipitated Mn(II) hydroxide is oxidised by oxygen to Mn(IV) oxo-hydroxide which is dissolved in mineral acid in presence of iodide:

$$Mn^{4+}\ (aq) + 3\ I^-(aq) \rightarrow Mn^{2+}(aq) + I_3^-\ (aq)$$

Then iodine formed (as triiodide ion I_3^-) can be titrated by sodium thiosulphate titrant solution of known concentration:

$$2\ S_2O_3^{2-}\ (aq) + I_3^- \rightarrow S_4O_6^{2-}\ (aq) + 3\ I^-\ (aq)$$

Finally, the concentration of dissolved oxygen can be calculated as

$$1\ \text{mole of}\ O_2 \rightarrow 4\ \text{mole of}\ S_2O_3^{2-}$$

The solubility of oxygen in water is about 8 $mg.L^{-1}$, *i.e.* 8 ppm, for laboratory temperature and normal air pressure (parcial pressure of oxygen about 20.9 kPa)[4]. The advantage of this absolute analytical method is that it does not need any calibration, it is very simple and it gives reliable results.

- **Preparation of the stock solutions of copper(I)**

All the solutions should be prepared and handled in an inert atmosphere (glovebox or Schlenk vessel/vacuum line). The stock solutions of copper(I) are commonly prepared by dissolving

tetrakis(acetonitrile)copper(I) tetrafluoroborate, hexafluorophosphate, perchlorate or triflate in degassed 100% acetonitrile (prepared accordingly to the above procedure).

Alternatively, copper(I) stock solutions can be prepared by dissolving copper(I) choride in an acidified (0.1 M hydrochloric acid) 1 M sodium chloride solution previously degassed (see above procedure).[5] Although Moffet *et al.* used 1 M sodium chloride, we found that copper(I) was slowly oxidizing under these conditions and sodium chloride concentration was increased to 4 M in order to achieve more stable copper(I) stock solution.

It is also possible to prepare the copper(I) stock solutions using a disproportionation reaction.[6] Namely, a 50 mM copper(I) stock solution can be prepared by incubating for 3 h under anaerobic conditions a 1 mL of degassed aqueous solution containing 25.0 mM $CuCl_2$, 1.0 g copper wire and 2-4 M acetonitrile previously degassed (acetonitrile is needed to stabilized the formed copper(I)). It is important to use a clean copper wire. In case of potential contamination of metal ions on the surface, it is recommended to clean the copper wire prior to use following the next protocol: the copper wire is incubated for 2 h with ethylenediaminetetraacetic acid (EDTA) under anaerobic conditions, through washed with 18.2 MΩ·cm Milli-Q water, dried and kept it in the glovebox (or anaerobic conditions).

Once prepared, it is crucial to keep the Cu(I) stock solutions under strict anaerobic conditions (glovebox). If not feasible, it is then recommended to prepare them freshly.

- **Determination of copper(I) ion concentration in the stock solutions**

The concentration of copper(I) can be determined using relative methods based on molecular absorption spectroscopy via the complexation with UV-VIS absorption metallochromic indicators such as bathocuproinedisulfonate (BCS; 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline disulfonic acid) or 2,2'-bicinchoninic acid (BCA) as follows:

- dissolve BCS or BCA (ca. 5 mM final concentration) in degassed water
- from the stock copper(I) solutions, prepare three samples with a 4-fold excess of either BCS or BCA at the µM range to ensure the formation of the respective colored $[Cu(BCS)_2]^{3-}$ and $[Cu(BCA)_2]^{3-}$ species. Given the molar absorbance of the Cu(I)/indicators adducts (see Tables below) the use of samples containing approx. 20 µM Cu(I) and 80 µM ligand) is suggested. These samples can be prepared in different buffer conditions (see Tables below). Prior to the addition of BCS or BCA, ethylenediaminetetraacetic acid (EDTA) can be added to the samples, at similar or slightly excess concentrations as the ligands, to prevent interference that might arise from the present of copper(II) (if any).[7]
- record the visible spectrum in a 1 cm quartz cuvette and use the absorbance at the right wavelength to determine the copper(I) concentration using the

corresponding extinction coefficient (see Tables below): Take the average concentration determined from the three samples.

Table 1. Spectral absorption features of $[Cu(I)(BCS)_2]^{3-}$ in aqueous solution.

λ_{max} (nm)	ε at λ_{max} (M^{-1} cm^{-1})	Medium conditions	Ref.
483	13300	20 mM Tris/MES; pH 8.*a*	[8]
483	12250	Hydroxylamine hydrochloride as reductant, pH range: 3 – 10.5	[9]
483	13000	20 mM phosphate; 100 mM NaCl; DMSO 30% *v/v*; pH 7 (buffer A, see reference)	[10]
482	13350	100 mM HEPES; 10 mM ascorbate; pH 7.4.*b*	[11]

a. Not precisely specified. Those reported are the conditions at which Cu(I)/BCS titrations with proteins were carried out; *b*. Spectral absorption features were determined also for the $[Cu(I)(BCS)]^{-}$ species (λ_{max} = 482 nm, $\varepsilon^{482\ nm}$ = 3830 M^{-1} cm^{-1}), see ref. [11]

Table 2. Spectral absorption features of $[Cu(I)(BCA)_2]^{3-}$ in aqueous solution.

λ_{max} (nm)	ε at λ_{max} (M^{-1} cm^{-1})	Medium conditions	Ref.
562*a*	7900	20 mM phosphate; 100 mM NaCl; DMSO 30% *v/v*; pH 7 (buffer A, see reference)	[10,12]
562	7700	50 mM HEPES; 200 mM NaCl; 0.20 mM ascorbate; pH 7.5	[13]
559	7840	100 mM HEPES; 10 mM ascorbate; pH 7.4.*b*	[11]

a. A maximum in the UV range was also reported, with $\boldsymbol{\lambda_{max}}$ = 358 nm and $\varepsilon^{358\ nm}$ = 42900 M^{-1} cm^{-1}. ; *b*. Spectral absorption features were determined also for the $[Cu(I)(BCA)]^{-}$ species (λ_{max} = 560 nm, $\varepsilon^{560\ nm}$ = 2300 M^{-1} cm^{-1}), see ref. [11]

- **Equilibrium constants for Cu(I) hydrolysis**

Metal ion hydrolysis is an important process that should be considered when determining the formation constants of copper(I) complexes. Copper(I) hydrolytic species are believed to be [Cu(OH)] and $[Cu(OH)_2]^{-}$.[14] The hydrolysis constants of these forms for zero ionic strength are collected in the Periodic Table prepared by the members of the WG1, and can be accessed through the link: https://www.cost-nectar.eu/pages/wg1_period.html.

Various approaches can be used to predict values at different ionic strengths and in different ionic media (e.g., Extended Debye-Hückel, Specific ion Interaction Theory (SIT) and Pitzer

models). The final selection criterion should be based on the availability of proper parameters. More at: I. Grenthe, I. Puigdomenech, *Modelling in Aquatic Chemistry*, Paris: OECD, 1997.

Depending on the experimental conditions used in the determination of the formation constants of copper(I) complexes, other equilibria should be considered (e.g. acetonitrile-Cu(I), chloride-Cu(I), ...). The members of the WG1 are currrently preparing a review on this topic.

References:

1. F. Rouessac, A. Rouessac, *Chemical analysis: modern instrumentation methods and techniques*, 2nd ed., Hoboken, NJ: John Wiley & Sons, 2007
2. D.C. Harris, C. A. Lucy, *Quantitative chemical analysis*. 10th ed. New York: W.H. Freeman and Company, 2020
3. Standard Methods for the Examination of Water and Wastewater. 15 Ed. American Public Health Association, Washington 1980.
4. a) G. A. Truesdale, A. L. Downing, G. F. Lowden, "The solubility of oxygen in pure water and sea-water". *J. Appl. Chem.*, 1955, 5(2), 53–62. https://doi.org/10.1002/jctb.5010050201. b) H. A. C. Montgomery, N. S. Thom, A. Cockburn, "Determination of dissolved oxygen by the winkler method and the solubility of oxygen in pure water and sea water". *J. Appl. Chem.* 1964, 14(7), 280–296. https://doi.org/10.1002/jctb.5010140704.
5. J.W. Moffet, R.G. Zika, R.G. Petasne, "Evaluation of bathocuproine for the spectrophotometric determination of Cu(I) in copper redox studies with applications in studies of natural waters". *Anal. Chim. Acta* 1985, 175, 171–179. https://doi.org/10.1016/S0003-2670(00)82729-4.
6. D.K. Johnson, M.J Stevenson, Z.A. Almadidy, S.E Jenkins, D.E. Wilcox, N.E. Grossoehme, "Stabilization of Cu(I) for binding and calorimetric measurements in aqueous solution". *Dalton Trans.*, 2015, 44, 16494–16505. https://doi.org/10.1039/C5DT02689J.
7. D. Chen, N. Darabedian, Z. Li, T. Kai, D. Jiang, F. Zhou. "An improved Bathocuproine assay for accurate valence identification and quantification of copper bound by biomolecules". *Anal. Biochem.* 2016, 497, 27–35. https://doi.org/10.1016/j.ab.2015.12.014.
8. Z. Xiao, F. Loughlin, G.N. George, G.J. Howlett, A.G. Wedd, "C-Terminal Domain of the Membrane Copper Transporter Ctr1 from Saccharomyces cerevisiae Binds Four Cu(I) Ions as a Cuprous-Thiolate Polynuclear Cluster: Sub-femtomolar Cu(I) Affinity of Three Proteins Involved in Copper Trafficking". *J. Am. Chem. Soc.* 2004, 126 (10), 3081-3090. https://doi.org/10.1021/ja0390350.
9. D. Blair, H. Diehl, "Bathophenanthrolinedisulphonic acid and bathocuproinedisulphonic acid, water soluble reagents for iron and copper". *Talanta*, 1961, 7 163–174. https://doi.org/10.1016/0039-9140(61)80006-4.
10. Z. Xiao, P.S. Donnelly, M. Zimmermann, A.G. Wedd, "Transfer of Copper between Bis(thiosemicarbazone) Ligands and Intracellular Copper-Binding Proteins. Insights into Mechanisms of Copper Uptake and Hypoxia Selectivity". *Inorg. Chem.* 2008, 47, 4338–4347. https://doi.org/10.1021/ic702440e.
11. M. Quaretti, M. Porchia, F. Tisato, A. Trapananti, G. Aquilanti, M. Damjanović, L. Marchiò, M. Giorgetti, M. Tegoni, "Thermodynamic stability and structure in aqueous solution of the [Cu(PTA)4]+ complex (PTA = aminophosphine-1,3,5-triaza-7-phosphaadamantane)". *J. Inorg. Biochem.* 2018, 188, 50–61. https://doi.org/10.1016/j.jinorgbio.2018.08.008.

12. K.Y. Djoko, Z. Xiao, D.L. Huffman, A.G. Wedd, “Conserved Mechanism of Copper Binding and Transfer. A Comparison of the Copper-Resistance Proteins PcoC from Escherichia coli and CopC from Pseudomonas syringae”. *Inorg. Chem.* 2007, 46, 4560–4568. https://doi.org/10.1021/ic070107o.
13. L.A. Yatsunyk, A.C. Rosenzweig, “Cu(I) Binding and Transfer by the N Terminus of the Wilson Disease Protein”. *J. Biol. Chem.* 2007, *282* (12), 8622–8631. https://doi.org/10.1074/jbc.M609533200.
14. P.L. Brown and C. Ekberg, Hydrolysis of Metal Ions. Wiley, 2016, pp. 650–702.