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Toxic metal sequential sequestration in water using new amido-aminoacid ligand as a model for the
interaction with polyamidoamines

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1 **Abstract**

2 Polyamidoamines are low cost and easily synthesized materials that may find applications in cations
3 sequestration and water treatment. In this paper a new amido-aminoacid ligand containing methionine
4 has been designed as a monomeric model of the corresponding polyamidoamine. The amido-aminoacid
5 ligand has been synthesized in high yield, by reacting acrylamide and methionine *via* aza-Michael
6 addition in water and mild temperature conditions. The reaction has been monitored by NMR and Raman
7 spectroscopies and the crystal structure has been determined by X-ray diffraction analysis. The
8 coordination ability of the ligand towards Cu^{2+} cations in water, as well as its affinity for Ni^{2+} and Co^{2+}
9 has been studied by potentiometric and spectrophotometric techniques. The divalent metal cations
10 sequestration from water may occur with sequential selection by changing the pH of the solution. The
11 copper complex with two coordinated ligands has been fully characterized in the solid state by single
12 crystal X-ray diffraction. The results are discussed with a view to use these materials in the treatment of
13 water contaminated by toxic transition metal ions.

14 **Keywords:** Sequential sequestration of transition metals; Amido-aminoacid ligand; Methionine;
15 Complex-formation equilibria; Wastewater treatments

16

17 **1. Introduction**

18 In the last decades, toxic heavy metals contamination in aqueous systems has become a global
19 environmental problem due to the harmful effects towards living organisms. Even at trace levels these
20 metals can cause ecological and health damages, due to their high toxicity, high solubility, migration
21 activity and stability in aqueous media and bioaccumulation tendency (Zheng et al., 2020; Essaimi et al.,
22 2012; Xiaoli et al., 2007; Marcovecchio, et al. 2007). High level of toxic heavy metals concentration in
23 water, as colloidal, particulate and dissolved phases, can originate from both natural (volcanic eruption
24 and rock weathering) and anthropogenic sources (solid waste disposal and agricultural, industrial or

25 domestic effluents) (Nasir et al., 2019; Vareda et al., 2019). The main toxic heavy metals found in aqueous
26 solutions are Cu, Cd, Hg, Pb, Ni and Zn (as divalent ions) and Cr, As and Se (multivalent ions) (Vardhan
27 et al., 2019; Malik et al., 2019).

28 To remove toxic metal ions from water systems, several processes have been proposed:
29 photocatalysis, electrochemical treatments, chemical precipitation, ion exchange, adsorption,
30 coagulation-flocculation, membrane filtration, bioremediation (Betiha et al., 2020; Tahir et al., 2019;
31 Brahmi et al., 2018; Alberti et al., 2018; Lee et al., 2016; Rezanian et al., 2016; Fu and Wang, 2011; Pan
32 et al., 2007). Among these methods, adsorption is one the most efficient techniques thanks to the easy
33 tuning for different targets, versatility in design, low cost and recyclability for multiple re-use (Wadhawan
34 et al., 2020; Zhou et al., 2018; Lakherwal et al., 2014)

35 Many efforts have been made to develop highly selective non-toxic and biocompatible adsorbent
36 materials (Bo et al., 2020; Joseph et al., 2019; Li et al., 2018; Uddin, 2017; Febrianto et al., 2009; Bailey
37 et al., 1999). The effectiveness in removing heavy metals from contaminated aqueous media depends on
38 the density of adsorption sites and on the capacity to strongly link metals. Chelating agents containing
39 amines and carboxylate groups, such as aminoacids, aminopolycarboxylic acids and polyamidoamines
40 are known to form stable structures with metal cations (Wang et al., 2020; Nasir et al., 2019; Ju et al.,
41 2019; Lachowicz et al., 2018; Bergamonti et al., 2017a; Tarazona-Vasquez and Balbuena, 2005).

42 Polyamidoamines (PAAs) are a class of biocompatible and biodegradable linear or cross-linked
43 polymers which can be functionalized, for example with hydroxyl, carboxylic or siloxane groups, for
44 several applications (Bergamonti et al., 2017b; Girardi et al., 2016; Isca et al., 2016; Zintchenko et al.,
45 2011; Ferruti et al., 2002). PAAs, in free-linear, cross-linked and silica-grafted forms, are able to
46 coordinate transition metal ions, namely Cu^{2+} , Ni^{2+} , Co^{2+} (Bergamonti et al., 2019; Casolaro et al., 1998;
47 Ferruti et al., 1980): the formed complexes are stable in water as proved by potentiometric and
48 spectroscopic studies (Bergamonti et al., 2017a; Tarazona-Vasquez and Balbuena, 2005; Xu and Zhao,
49 2005; Ferruti et al., 1981; Barbucci et al., 1980). These results suggest the use of polyamidoamines as

50 inorganic pollutants sorbing materials for wastewater purification (Manfredi et al., 2013; Ferruti et al.,
51 2012).

52 PAAs are prepared by addition reaction between bisacrylamide and amines or aminoacids (Ferruti,
53 2013). This is known as aza-Michael addition and is regarded as a green reaction due to the use of water
54 as solvent, the mild temperature conditions and the absence of added catalysts.

55 Among the aza-Michael additions, the use of aminoacids as source of amines for the PAAs synthesis has
56 been scarcely investigated (Manfredi et al., 2017; Ferruti et al., 2014). Some studies of model
57 amidoamines in monomeric form derived from aminoacids have been reported (Nehls et al., 2013;
58 Chandrarekha et al., 2015) and few examples on the capability of these molecules to coordinate metal
59 cations (Rajalakshmi et al., 2008; Lim et al., 1994) have been described. Nevertheless, the use of
60 aminoacids allows the introduction, in the polymeric structure, of carboxylate functions which may be
61 significant in determining stable coordination of metal ions, and subsequent higher efficiency to strongly
62 adsorb metals.

63 Here we have characterized by spectroscopic and diffractometric methods the product of acrylamide
64 with methionine (AcryMet) as a monomeric molecular model of the corresponding polyamidoamine. The
65 coordination ability of this monomer toward Cu^{2+} and, for the sake of comparison, toward Co^{2+} and Ni^{2+} ,
66 has been evaluated in aqueous solution by studying their speciation models.

67 The results obtained for the AcryMet model are the starting point to assess the metal coordination
68 ability of the corresponding polymer for its use as specific absorber of heavy metal ions for water
69 purification. In particular, we will show here that AcryMet is able to form complexes with Cu^{2+} , Ni^{2+} and
70 Co^{2+} in different pH ranges. This behavior may be used to differentiate metal ions on the basis of their
71 sequential binding to these ligands and their polymers as the pH of the medium increases from acidic to
72 neutral.

73

74

75 2. Experimental Section

76 2.1 Materials

77 Acrylamide (98%), L-methionine (99.9%), copper (II) acetate (99%), nickel (II) acetate, cobalt (II)
78 acetate, DMSO- d_6 were purchased from Sigma-Aldrich and used as received. Concentrated sodium
79 hydroxide and Titrisol[®] solutions were purchased by Merck.

80 2.2 Synthesis of 3(2-amino-4-(methylthio)butanoic acid)propanamide (AcryMet).

81 A water solution of pure L-methionine was added to a water solution of acrylamide in equimolar
82 ratio in presence of ammonium buffer solution (pH=10.6). The reaction mixture was stirred at 70 °C for
83 4 hours and then the solvent was evaporated in vacuum to give the crude product (yield: 99%) as a white
84 microcrystalline powder. The powder was then re-dissolved in water and, after a slow evaporation of the
85 solvent at 4 °C, colorless crystals with a needle aspect of AcryMet were obtained, suitable for X ray
86 diffraction analysis.

87 2.3 Reaction of AcryMet with copper acetate. Synthesis of Cu-AcryMet complex

88 A water solution (15 mL) of AcryMet (0.176 g, 0.8 mmol) was added dropwise to a water solution
89 (15 mL) containing $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ (0.080 g, 0.4 mmol). The blue solution was stirred at room
90 temperature for 1 hour. Then the solvent was allowed to evaporate. The solid residue was re-dissolved in
91 water and, by slow crystallization using acetone as non-solvent, blue single crystals were obtained (Yield
92 70%).

93 2.4 Nuclear Magnetic Resonance

94 ¹H NMR spectra were recorded in a spectrometer JEOL ECZ600R operating at 600.17 MHz using a
95 5 mm multinuclear probe for high resolution with actively shielded gradient along the z-axis, equipped
96 for automatic tuning and matching. The acquisition of spectra was carried out using solutions of about 75
97 mg of the compound dissolved in 0.6 mL of deuterated solvent. The probe temperature was 318 K for all
98 spectra. The solvents used was DMSO- d_6 , used also as internal reference. The complete characterization

99 was done running 1D ^1H and ^{13}C DEPT spectra, 2D homonuclear COSY, TOCSY and heteronuclear
100 HSQC and HMBC spectra.

101 The typical operation conditions for the 1D proton spectra were: 18063.58 Hz spectral width with 64 K
102 data points, 32 scans: Acquisition time and repetition time were 3.63 and 6.63 sec, respectively.

103 Gradient-selected gCOSY (Hurd, 1990) spectra were obtained with 4096 data points covering a spectral
104 width of 9.0058 kHz in both dimensions. Two scans were acquired for each of 256 increments, with an
105 acquisition time of 0.568 s and a relaxation delay of 1.5 s.

106 TOCSY spectra (Levitt et al., 1982) were acquired with a spinlock time of 80 ms, with the same
107 experimental parameters reported for gCOSY. Four scans were done for each of 256 increments, with an
108 acquisition time of 0.455 s and a relaxation delay of 1.5 s.

109 Gradient-selected HSQC spectra (Wilker et al., 1993) were obtained with the same experimental
110 parameters reported for TOCSY in F2 and a spectral width of 25.668 kHz in F1.

111 The gradient selected heteronuclear multiple bond correlation spectra HMBC (Rinaldi and Keifer, 1994)
112 were obtained in the same conditions, with spectral widths of 37.764 kHz in F1.

113 *2.5 Raman Spectroscopy*

114 Raman spectra were acquired at 632.8 nm in backscattered geometry with a Horiba - Jobin Yvon
115 LabRam micro-spectrometer (300 mm focal length) equipped with an integrated Olympus BX40
116 microscope. The spectral resolution was about 1.5 cm^{-1} . The laser power on the samples was adjusted by
117 means of density filters to avoid uncontrolled thermal effects and always kept less than 1 mW. Spectra
118 were collected using a long working distance x50 microscope objective. Typical exposures were 60-90 s,
119 with 3-5 repetitions.

120 *2.6 Potentiometric studies*

121 The potentiometric titrations of ligand AcryMet (HL) were carried out in aqueous solution at $T =$
122 $298.2 \pm 0.1\text{ K}$ and $I = 0.1\text{ M}$ (KCl) under N_2 stream, using 20 mL samples. The potentiometric apparatus

123 was previously described (Quaretti et al., 2018). The Hamilton combined glass electrode (P/N 238000)
124 was calibrated in terms of $[H^+]$ by titrating HCl solutions with a 0.14 M carbonate-free standardized
125 solution of KOH and the pK_w value resulted to be 13.76(1). Protonation data were obtained by alkalimetric
126 titration of 3 samples ($C_{Ligand} = 2.8-8.8 \cdot 10^{-3}$ M). Formation constants of the complexes with the divalent
127 metals Cu^{2+} , Co^{2+} , Ni^{2+} , and Zn^{2+} (M^{2+}) were determined by alkalimetric titration of 3 samples ($C_{Metal} =$
128 $0.65-1.8 \cdot 10^{-3}$ M; L:M = 1.5-4.2). Systems containing Cu^{2+} and Ni^{2+} were studied between pH 2.5 and 11.
129 Systems containing Co^{2+} and Zn^{2+} were limited to the pH range 2.5-9 and 2-8, respectively. For the latter
130 two systems, drift in the e.m.f. and opalescence was observed at higher pH.

131 The protonation and complex-formation constants of the systems were calculated with HyperQuad
132 2013 software (Gans et al., 1996) and the results were used to draw the species distribution curves with
133 Hyss 2009 program (Alderighi et al., 1999).

134 *2.7 UV-visible spectrophotometry*

135 UV-visible spectra of the $Cu^{2+}/AcryMet$; $Ni^{2+}/AcryMet$ and $Co^{2+}/AcryMet$ systems were collected
136 with an Evolution 260 Bio (Thermo Scientific, Waltham, MA, USA) spectrophotometer provided with a
137 Peltier thermostat, using quartz cuvettes of 1 cm path length. Solutions containing the ligand (L) and the
138 metal (M) were prepared under inert atmosphere ($C_L = 5-5.5 \cdot 10^{-3}$ M, M:L = 2.2 for Cu^{2+} , or 4.2 for Co^{2+}
139 and Ni^{2+} ; total volume 20 mL). The pH was adjusted to selected pH values by additions of standard KOH
140 titrant, the solution transferred in the cuvette and the spectrum collected in the 250-900 nm range. The
141 range of pH was 2.1-11.2 for Cu^{2+} , 3.2-10.8 for Ni^{2+} , and 3.2-8.9 for Co^{2+} . At higher pH values, turbidity
142 in the solutions of $Ni^{2+}/AcryMet$ and $Co^{2+}/AcryMet$ was observed. UV-visible spectrophotometric
143 titration data for the $Cu^{2+}/AcryMet$ system were analysed with the HypSpec 2014 program (Gans et al.,
144 1996).

145 *2.8 Metal ion adsorption*

146 The metal ions adsorption capacity of Acry-Met ligand was determined by UV-Vis adsorption
147 spectrophotometry. To perform the adsorption experiments, the Acry-Met ligand was dispersed on a TEOS

148 (Tetraethyl orthosilicate) film prepared via sol-gel in basic medium: 0.9 g of Acry-Met (4.1 mmol; pH =
149 9.6) was dissolved in 5 ml of water and added to a TEOS solution, previously prepared solving 3.2 ml of
150 TEOS in 5 ml of ethanol; the resulting solution was cast in a Petri disk and left drying at room temperature
151 24h to obtain the film; the film was equally cut in three part. Then, 20 ml aqueous solutions (0.0125
152 mol/L) of Cu (II), Co (II) and Ni (II) were prepared starting from metal acetates. Acry-Met ligand film
153 was added to every metal ion solution, obtaining Lig/M²⁺ = 5:1. The initial pH of the solutions was
154 adjusted with 0.1 M HCl (pH_{Cu} = 4.5, pH_{Co,Ni} = 6). At defined time intervals, 0.5 ml of solution were
155 taking and filtered (pore size 0.45µm). Absorbance of metal ion solution was determined at the maximum
156 absorption wavelength (712 for Cu(II), 514 nm for Co(II) and for Ni(II)) by UV-Vis. The experiments
157 were carried out at room temperature. Adsorption experiment, as a blank correction, was performed on
158 pure TEOS film.

159 To determine the adsorption capacity (adsorption uptake rate) of adsorbent at equilibrium, q_e (mg/g), and
160 the removal percentage, R_{ads} (%), due to adsorption of metal ions, the following equations was used:

$$161 \quad q_e = \frac{C_0 - C_e}{w} V \quad (1)$$

$$162 \quad R_{ads} (\%) = \frac{C_0 - C_e}{C_0} 100 \quad (2)$$

163 where C_0 (mg/L) and C_e (mg/L) are the initial and equilibrium concentration of metal solution,
164 respectively. V (ml) is the volume of adsorbate solution and w (g) is the weight of adsorbent.

166 2.9 X-ray Data Collection, structure solution and refinement of AcryMet and Cu²⁺/AcryMet complex.

167 X-ray crystallographic data of AcryMet and Cu²⁺/AcryMet complex were obtained on single crystals
168 with an APEX 2 Bruker CCD diffractometer. The APEX 3 program package (Bruker, 2015) was used for
169 the data collection (30 s/frame scan time for a sphere of diffraction data) and to determine the unit-cell
170 parameters. The structures were solved using SHELXT (Sheldrick, 2015) by Intrinsic Phasing method in
171 the APEX 3 program. Subsequent calculations were carried out using the SHELXTL-2014/7 program in
172 the WinGX suite v.2014.1 (Farrugia, 2012). The refinement was carried out based on F² by full-matrix
173 least-squares techniques. Crystallographic data have been deposited with the Cambridge Crystallographic
174 Data Centre as supplementary publication. Copies of the data can be obtained free of charge on application
175 to the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax, (+44) 1223 336033; e-mail,

176 deposit@ccdc. cam.ac.uk). Deposition Number CCDC 2023768 and 2023769 for AcryMet
177 and Cu²⁺/AcryMet respectively.

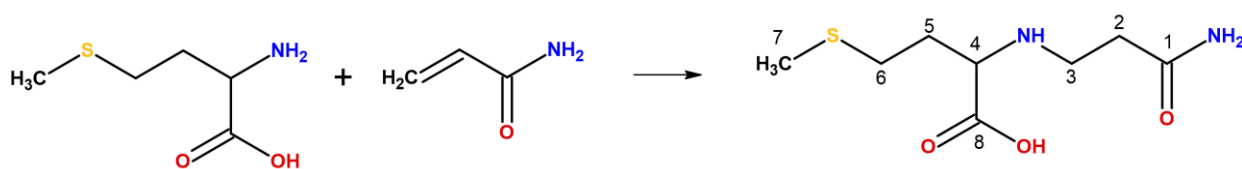
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179 3. Results and Discussion

180 3.1 NMR and Raman spectroscopic characterization of AcryMet.

181 The addition between acrylamide and L-methionine in equimolar ratio leads to the formation in high
182 yield (99 %) of the AcryMet adduct, whose structure is reported in Scheme 1.

183



185 **Scheme 1.** Addition reaction between L-methionine and acrylamide, leading to the formation of AcryMet adduct.

186

187 The addition reaction can be monitored by NMR and Raman spectroscopies, all evidencing the
188 disappearance of the double bond typical of the acrylamide molecule.

189 ¹H and ¹³C chemical shifts in DMSO-*d*₆ solution are reported in Table 1. The protons and carbons
190 chemical shift were assigned by mono- and bi-dimensional spectra recorded at 318 K. Figure 1 shows the
191 proton spectra, expansion 1D and 2D TOCSY, and Figure 2 the 2D hetero-correlated proton-carbon
192 spectra HSQC (violet) and HMBC (green). HSQC correlates the carbons to directly linked protons (see
193 Table 1). DEPT spectrum, shown as Y projection, combined with HSQC spectrum clearly displays the
194 presence of six carbons, four methylene, one methine and one methyl groups.

195 The two carbonyls carbon atoms C1 (174.32 ppm) and C8 (178.57ppm) were assigned by HMBC
196 spectrum, by mean of their long-range correlation with H2, H3 and H4, H5, respectively.

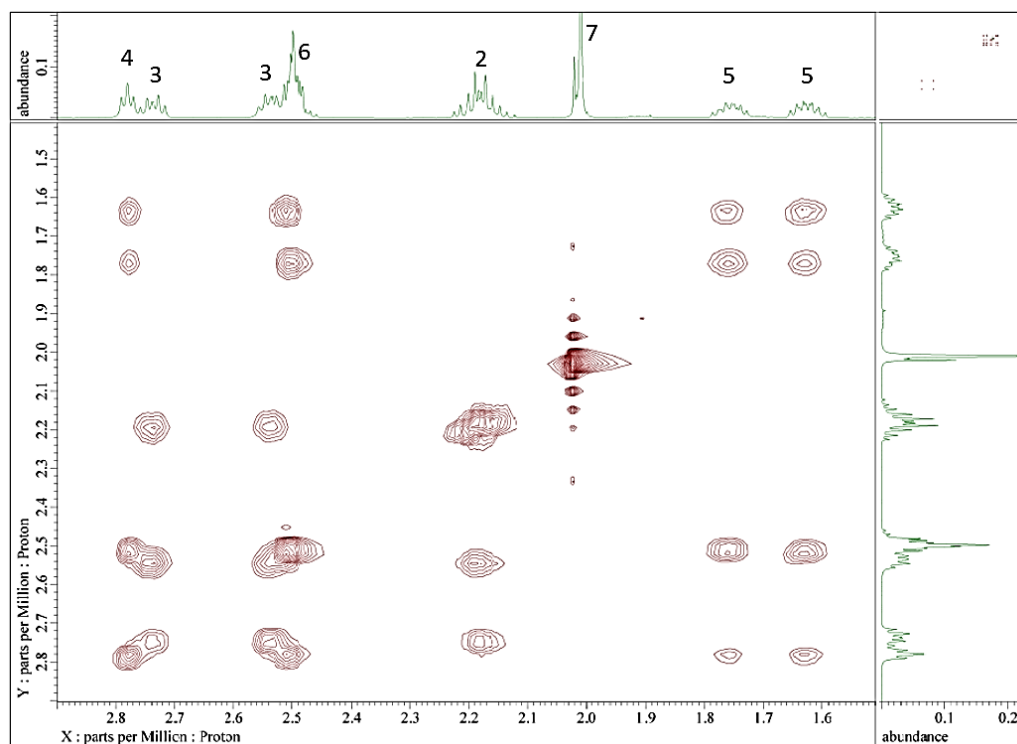
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Table 1. ^1H and ^{13}C NMR chemical shifts for AcryMet

Atoms identification (Scheme 1)	^1H ppm	^{13}C ppm
1		174.32
2	2.13	35.73
3	2.49; 2.68	44.21
4	2.72	62.41
5	1.58; 1.71	33.09
6	2.46	30.33
7	2.00	14.30
8		178.57
NH	7.58	
NH ₂	6.64	

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202

Fig. 1. 1D and 2D TOCSY spectra for compound AcryMet

203

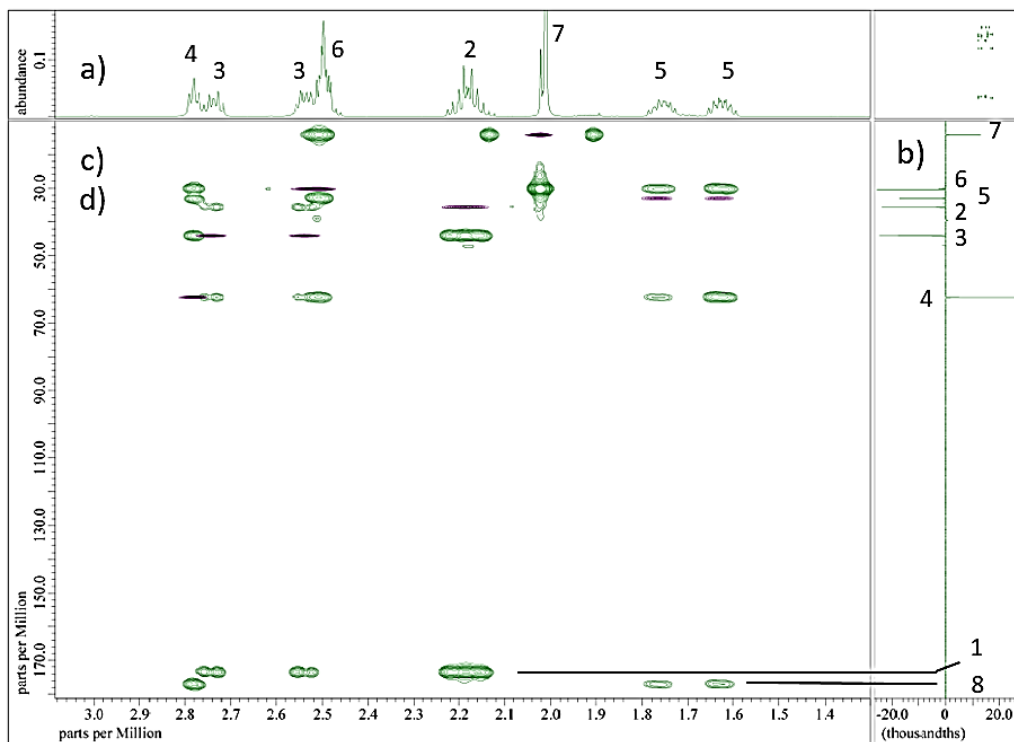


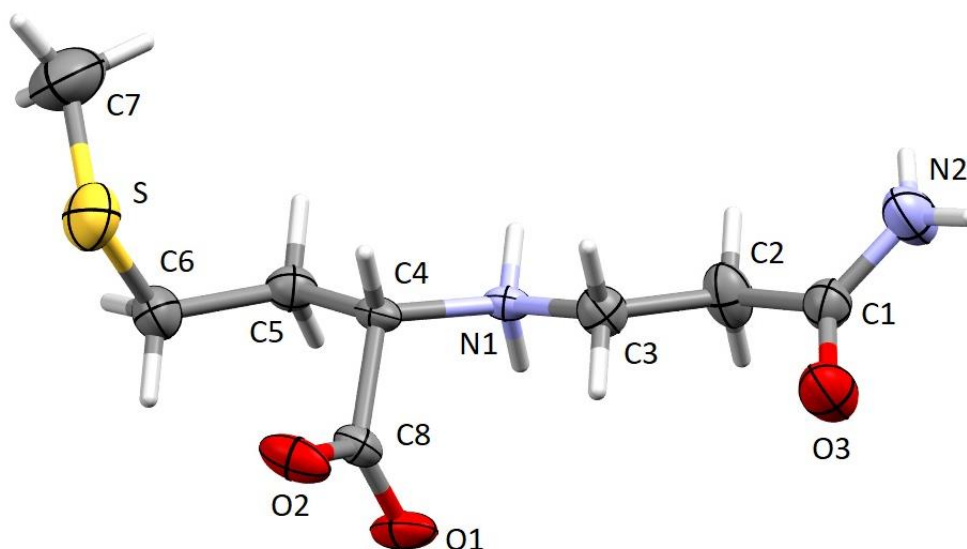
Fig. 2. 1D ^1H (a), ^{13}C DEPT (b), 2D HSQC ((c), violet) and HMBC ((d), green) spectra for compound AcryMet.

Raman (Figure S1) spectra of the starting reagents and of the powdered final product AcryMet were recorded. In the Raman spectrum of acrylamide, the peaks due to vibrational modes of the amide group and of the double bond are very strong: the C=O stretching at 1681 cm^{-1} , the NH_2 deformation at 1583 cm^{-1} and the C=C stretching vibration ($\nu\text{C}=\text{C}$) at 1630 cm^{-1} . Due to the chemical changes that occur during the reaction, in the final product the amide I peak down-shifts at 1663 cm^{-1} , the amide II up-shifts to 1605 cm^{-1} and the $\nu\text{C}=\text{C}$ band disappears (Bergamonti et al., 2017a; Duarte et al., 2005). confirming that the double bond addition reaction has occurred.

3.2 Crystal structure of AcryMet

The molecule presents the expected zwitterionic form in its crystalline phase (Figure 3) as evidenced by the near equal distances C-O observed in the carboxylic moiety [C8-O2 1.236(3) and C8-O1 1.248(3) Å]. The two carboxylate oxygen atoms O1 and O2 participate in strong head to tail $\text{O}\cdots\text{H}-\text{N}$ hydrogen

219 bonds with protonated $RR'NH^{2+}$ groups of adjacent molecules, and these interactions lead to a three
220 dimensional network [N1-H1b \cdots O1 2.714(2) Å, N1-H1b \cdots O1 173.54(1)° and N1-H1a \cdots O2 2.767(3) Å,
221 N1-H1a \cdots O2 149.64(1)°]. Moreover, the carbamoyl groups of the molecule form N-H \cdots O hydrogen
222 bonds within themselves, [N2-H2c \cdots O3 3.010(3) Å, N2-H2c \cdots O3 167.02(1)°], leading to chain linked
223 molecules, developing along the *a* axis, as shown in Figure 4. This causes a preferred direction in the
224 crystal growth along this axis, and the formation of needle form crystals. The molecule is enantiomeric,
225 as derived from L-methionine and it crystallizes in a chiral space group $P2_12_12_1$ with *S* configuration.
226



227
228 **Fig. 3.** Ortep view of AcryMet. Hydrogen atoms are drawn in capped stick style.
229
230
231
232

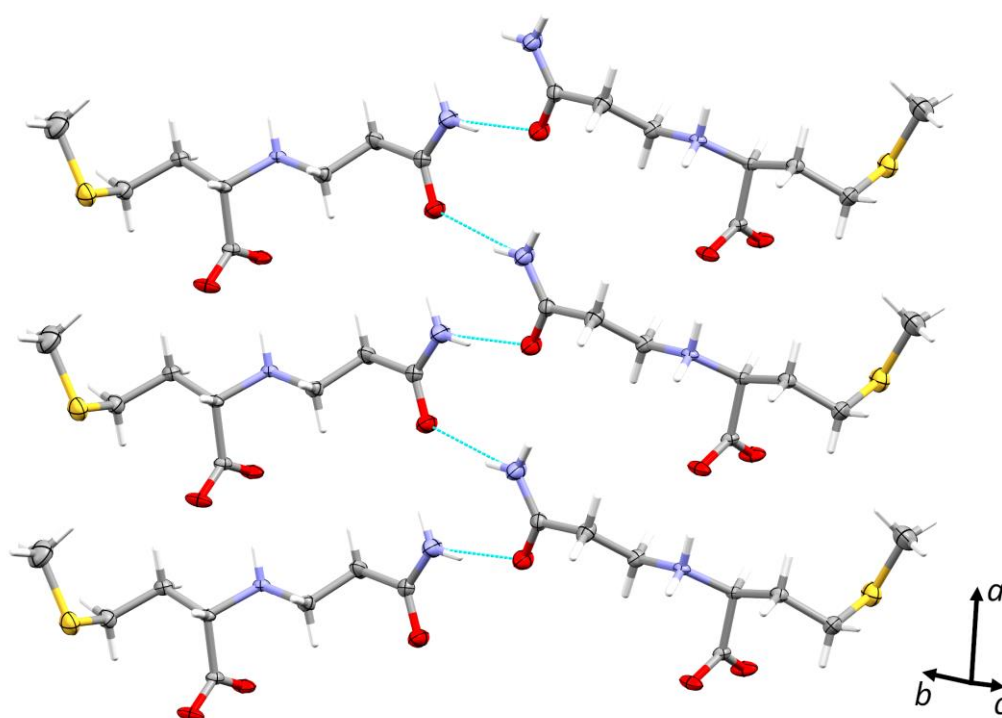


Fig. 4. Carbamoyl group forming N-H...O hydrogen bonds leading to chains linking related molecules, developing along the crystallographic *a* axis.

The anisotropic nature of the AcryMet crystals is confirmed by the Raman spectra on a single needle-like crystal taken at perpendicular orientations, as shown in figure S2. Because of hydrogen bonds play an important roles in the stability of the AcryMet crystalline structure, the main changes in the the vibrational spectrum are linked to the modes of groups involved in the H bonds, as clearly evident in the regions of the NH stretching and bending vibrations at $3150\text{-}3500\text{ cm}^{-1}$ and $1500\text{-}1600\text{ cm}^{-1}$, respectively.

3.3 Formation of copper complex $\text{Cu}^{2+}/\text{AcryMet}$ in solution and fully characterization in solid state

AcryMet has been reacted with Cu^{2+} cations in order to test its ability to coordinate metal ions in wastewater. In fact, the molecule presents three potential sites with high electron density, capable to coordinate to metal ions: (a) the carboxylate group, (b) the bis-amine site and, upon deprotonation, (c) the

246 amide carbonyl group. The study of the coordination ability can be of great interest in order to use the
247 corresponding polymer (PAA) as active material able to sequester metal ions from wastewater.

248 The molecular view of the Cu²⁺/AcryMet complex is shown in Figure 5. L and D enantiomers of the
249 amido aminoacid coordinate the metal cation. Coordination of Cu²⁺ ions was reported in the literature to
250 promote racemization of amino acids (Mathews and H. Manohar, 1991; Weinstein et al., 1970; Byun et
251 al., 2017; Gillard and O'Brien, 1978). However, in these processes the formation of a Schiff base at the
252 amino group makes the α -carbon more acidic. For AcryMet this is not necessary and only the presence of
253 the copper ion induces racemization, probably for the strong interaction of the metal with the tridentate
254 ligand.

255 The copper atom is placed on an inversion center and the complex shows a Jahn-Teller distorted
256 octahedral environment, achieved by the nitrogen atom of the aminic group and the oxygen atoms of the
257 carboxylic group on the equatorial plane, evidencing closer interactions [Cu-N1 2.008(9) Å, Cu-O1
258 1.909(7) Å] and of the amidic moieties of the ligand in the apical position at longer distance [Cu-O3
259 2.569(8) Å]. Each molecule acts as a tridentate ligand, forming a five membered chelating ring through
260 the carboxylic and aminic groups and a six membered chelating ring through the aminic and amidic
261 moieties; the tio-alkyl chain remains dangling and not involved in interactions. We anticipate here that
262 1:2 Cu²⁺:ligand species is formed in solution for which we propose the same equatorial coordination (2
263 NHR, 2 COO⁻) environment (see below).

264

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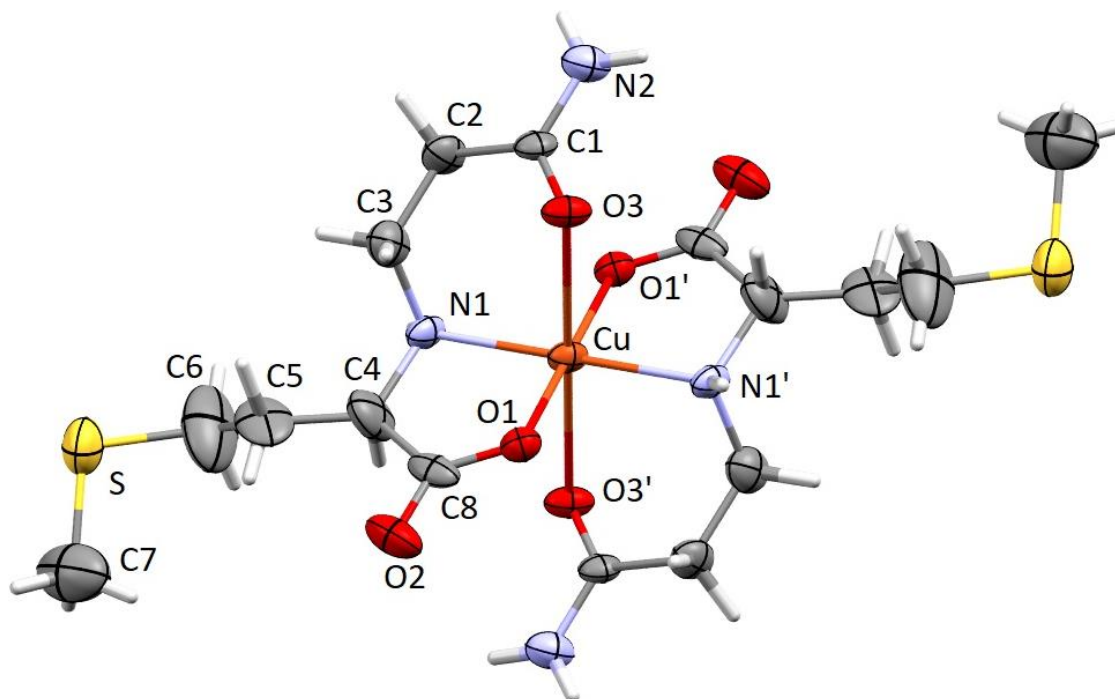


Fig. 5. Ortep view of Cu²⁺/AcryMet Complex.

3.4 Solution equilibria

In the perspective of using AcryMet-based polymers for the extraction of metal ions from aqueous solutions, we have studied the Cu²⁺ coordination capabilities of AcryMet in water. These studies were carried out by potentiometry and UV-visible absorption spectrophotometry, and they allowed to determine the speciation of the Cu²⁺/AcryMet system, which is reported in Table 2. The speciation of the Co²⁺, Ni²⁺ and Zn²⁺ / AcryMet systems were determined for the sake of comparison.

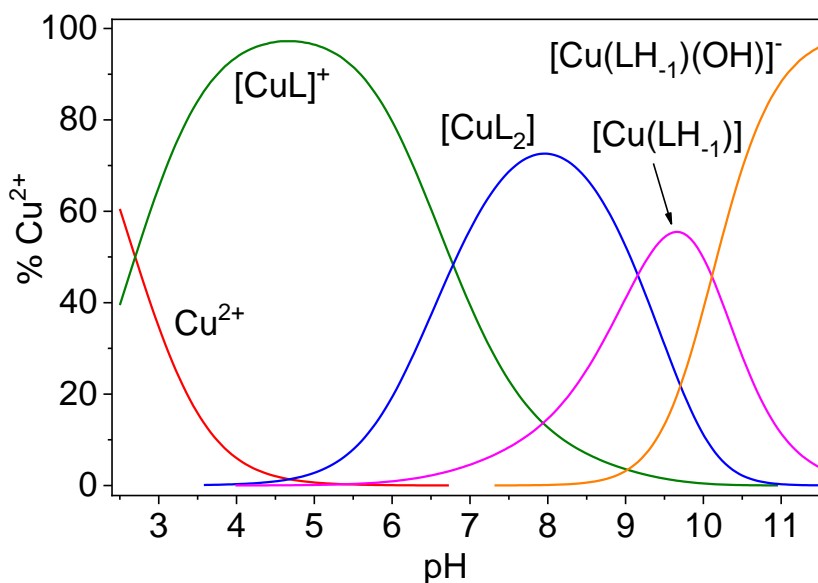
Table 2. Logarithms of formation constants of the complex species of Cu²⁺, Co²⁺, Ni²⁺ and Zn²⁺ with AcryMet in aqueous solution ($I = 0.1$ M (KCl), $T = 298.2$ K). Visible absorption maxima of the Cu²⁺/AcryMet species are also reported.

	Cu ²⁺		Co ²⁺	Ni ²⁺	Zn ²⁺
	log β	λ _{max} (nm)	log β	log β	log β
[ML] ⁺	7.99(1)	712 (717)	3.93(1)	5.52(9)	3.67(1)
[ML ₂]	12.28(1)	628 (622)	5.99(6)	8.90(1)	5.97(7)
[MLH ₋₁]	0.03(1)	633 (627)	-	-5.86(7)	-
[MLH ₋₂] ⁻	-10.06(1) ^a	616 (592)	-	-	-
σ	1.27		2.60	2.48	3.50
n	256		139	169	144

^a For this species we propose a [Cu(LH₋₁)(OH)]⁻ stoichiometry, see text.

AcryMet in aqueous solution behaves as a biprotic ligand. In its fully protonated form (H₂L⁺), AcryMet is protonated on both the carboxyl- and secondary amino functions. The two groups have pK_a of 1.47(2) and 7.99(1), respectively. These results confirm that AcryMet in solution is zwitterionic in its neutral form HL, in accordance with the crystal structure.

The ligand forms with Cu²⁺ four complex species: [CuL]⁺, [CuL₂], [Cu(LH₋₁)] and [Cu(LH₋₁)(OH)]⁻. The logarithms of their formation constants are reported in Table 2, and a distribution diagram is represented in Figure 6. Cu²⁺ complexation starts below pH = 3: at pH 2.4, where we started to collect potentiometric data, ca. 30 % total copper results already in the [CuL]⁺ form. The [CuL]⁺ complex reaches 95 % total copper at pH 4.8. The [CuL₂] species starts to form at pH 5 and dominates between pH 6.8 and 9.2 with a maximum of 75% total copper at pH 8. The [Cu(LH₋₁)] species starts to form at pH 7, followed by the formation of [Cu(LH₋₁)(OH)]⁻. The structural models that we put forward for these species are reported in Scheme 2 and are well supported by the UV-visible absorption features that are discussed here below.



296

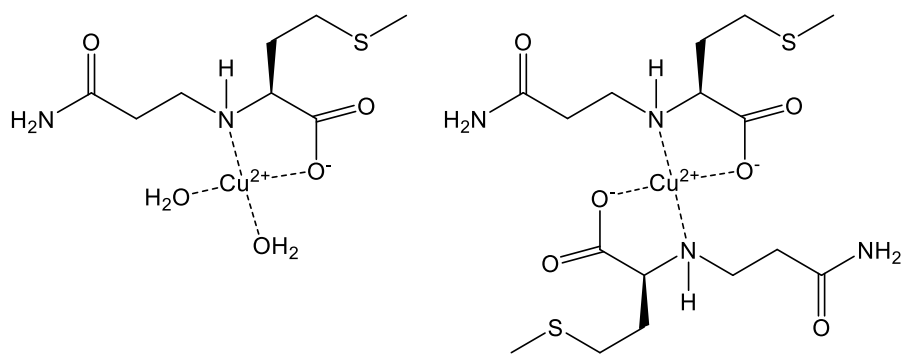
297 **Fig. 6.** Representative distribution diagram of the Cu^{2+} /AcryMet (HL) system ($\text{Cu}:\text{L} = 1:2.2$; $C_{\text{Cu}} = 0.0125 \text{ M}$) in
 298 aqueous solution. $I = 0.1 \text{ M}$ (KCl), $T = 298.2 \text{ K}$.

299

300 Knowing the speciation system, we have investigated the Cu^{2+}/L system by UV-visible titration as a
 301 function of the pH. The spectral dataset is reported in Figure S3. On the basis of these spectral data we
 302 have calculated the UV-visible molar spectra of the Cu^{2+} species, which are reported in Figure S4. The
 303 wavelengths corresponding to the absorption maxima of the different complex species are reported in
 304 Table 2.

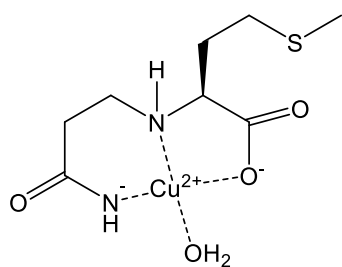
305 The schematic structures of the proposed equatorial coordination environment of the Cu^{2+} species
 306 are represented in Scheme 2. The expected wavelengths of the absorption maxima of these species were
 307 calculated using the rule of the average environment (Billo, 1974; Prenesti et al., 1999) and their values
 308 are also reported in Table 2.

309

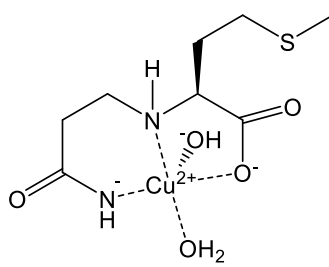


[CuL]⁺

[CuL₂]



[Cu(LH₋₁)]



[Cu(LH₋₁)(OH)]⁻

310

311 **Scheme 2.** Schematic representation of the equatorial coordination environment of the [CuL]⁺, [CuL₂], [Cu(LH₋₁)]

312 and [Cu(LH₋₁)(OH)]⁻ species.

313

314 The evaluation of the spectral data (λ_{max} values in particular, Table 2) suggests that the AcryMet

315 ligand acts as a bidentate (NHR, COO⁻) chelating ligand in [CuL]⁺ and [CuL₂]. This observation is very

316 consistent with the crystal structure of Cu²⁺/AcryMet. Actually, the distorted octahedral coordination of

317 Cu²⁺ has the amino and carboxylate groups as the equatorial donor atoms, as we propose in solution for

318 the 1:2 metal:ligand complex. Absorption data do not provide indications on the nature of the axial ligand.

319 Although amide oxygen atoms can coordinate to axial positions, we put forward the hypothesis that these
320 weak interactions are rather substituted by water coordination.

321 At pH higher than 5 the deprotonation of the amidic nitrogen occurs, with formation of $[\text{Cu}(\text{LH}_{-1})]$,
322 where the ligand acts as a tridentate (N^- , NHR , COO^-) chelating molecule. The deprotonation of the amidic
323 nitrogen is prompted by the formation, in $[\text{Cu}(\text{LH}_{-1})]$, of a 5- and 6 membered chelating rings. For all
324 three $[\text{CuL}]^+$, $[\text{CuL}_2]$, and $[\text{Cu}(\text{LH}_{-1})]$ species the observed wavelengths corresponding to the absorption
325 maxima are consistent with the coordination modes proposed in Scheme 2.

326 Since no other acidic protons are present in the ligand, the highest deprotonated $[\text{Cu}(\text{LH}_{-1})(\text{OH})]^-$
327 species corresponds to an hydroxo-coordinated complex (Scheme 2). Spectroscopic and potentiometric
328 data suggest that a mixture of axially- and equatorially-deprotonated water molecules are present, or more
329 likely a distorted species which is structurally intermediate between the two geometries. On one hand, the
330 $\text{p}K_a$ of the $[\text{Cu}(\text{LH}_{-1})] = [\text{Cu}(\text{LH}_{-1})(\text{OH})]^- + \text{H}^+$ process results -10.03(1). This value is intermediate
331 between that of $[\text{CuL}]$ of trien (10.7) where the deprotonation of an axial water molecule takes place, and
332 that of $[\text{CuL}]$ of dien (9.1) where the deprotonation is certainly on the equatorial plane (Smith et al., 2007).
333 Also, should the deprotonation of $[\text{Cu}(\text{LH}_{-1})]$ occur on the equatorial plane, a blue shift to ca. 595 nm is
334 expected on the basis of the Billo's parameters (Prenesti et al., 1999). Conversely, a small red shift is
335 expected for the formation of an axially-coordinated hydroxo-complex (Sigel and Martin, 1982).
336 Therefore, the experimental λ_{max} of 616 nm determined for $[\text{Cu}(\text{LH}_{-1})(\text{OH})]^-$ is intermediate between the
337 spectral shifts expected for the deprotonation of $[\text{Cu}(\text{LH}_{-1})]$ into either an axially- and an equatorially-
338 bound hydroxide. One last experimental evidence for the presence of an intermediate axial-equatorial
339 hydroxo-species relates with the spectrum bandwidth. The calculated spectra in Figure S5 show no
340 appreciable broadening effect or maximum splitting for the calculated spectrum of $[\text{Cu}(\text{LH}_{-1})(\text{OH})]^-$
341 compared to those of the other species. In this respect, if two different species absorbing at ca. 595 and
342 620 nm, respectively, are present in solution, a significant band broadening should be observed. This was
343 not evidenced experimentally, and therefore we conclude that $[\text{Cu}(\text{LH}_{-1})(\text{OH})]^-$ corresponds to one single
344 species geometrically intermediate between and axially- and an equatorially- hydroxo-complex.

345 Potentiometric and spectrophotometric studies were carried out also on the Ni²⁺, Co²⁺ and
346 Zn²⁺/AcryMet systems. In particular we determined the stability of the complexes of Ni²⁺ and Co²⁺ with
347 the purpose of finding conditions that allow to control their binding to the ligand. Logarithms of complex
348 formation constants are reported in Table 2, while representative speciation diagrams are reported as
349 supporting information. Spectra dataset for the UV-vis characterization of systems containing Ni²⁺ and
350 Co²⁺ as a function of the pH are also reported as supporting info. For all three cations both [ML]⁺ and
351 [ML₂] species form in solution. For Ni²⁺, a small amount of [Ni(LH₋₁)] is also formed at high pH values.
352 For all these complexes, the proposed coordination modes of AcryMet in solution are analogous to those
353 of the complexes of Cu²⁺, considering the species with the same stoichiometry.

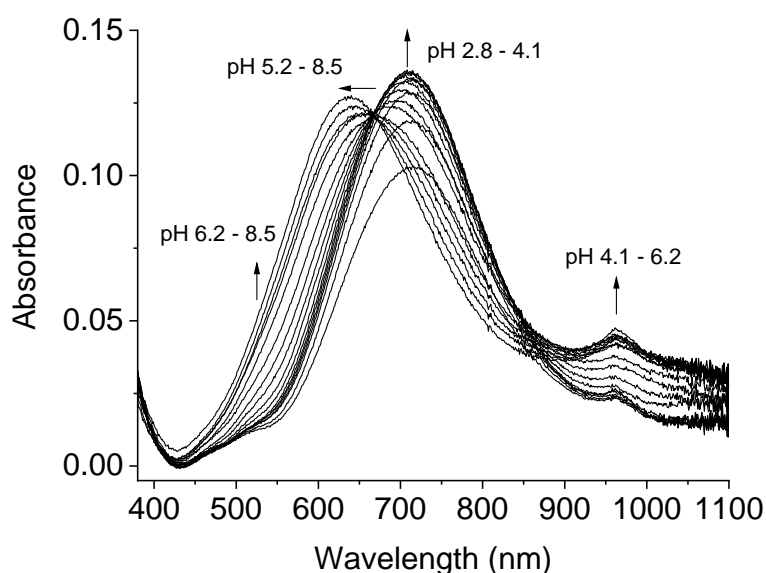
354 Quite expectedly, data in Table 2 (e.g. log β of [ML]⁺ or [ML₂] species) evidence that the order of
355 the global formation constants reflects the Irving-Williams series, (i.e. Co²⁺ < Ni²⁺ < Cu²⁺ > Zn²⁺). This
356 behavior is also reflected into the pH at which, for instance, the [ML]⁺ species is formed. Cu²⁺ forms the
357 most stable complexes, and in fact 30% total copper is already in the [CuL]⁺ form at pH 2.5. On the
358 contrary, Ni²⁺ complexes start to form at pH 3.5, Co²⁺ species at pH 3.5, and Zn²⁺ species at pH 5.5. It is
359 therefore clear that through a wise choice of the pH conditions of the medium it is possible to obtain
360 solely Cu²⁺ coordinated to the ligand (low pH), or Cu²⁺ and Ni²⁺ (intermediate pH), or all three Cu²⁺, Ni²⁺
361 and Co²⁺ cations (high pH).

362 In the perspective of using AcryMet-based polymers to sequester toxic transition metal ions from
363 water, we have therefore calculated a competition distribution diagram for a theoretical
364 Co²⁺/Ni²⁺/Cu²⁺/MAA (HL) = 1:1:1:3 system, represented in Figure S6-11. In this system, L is expected
365 to bind almost selectively Cu²⁺ at pH below 4. Between pH 4 and 6, binding of both Cu²⁺ and Ni²⁺ is
366 expected, while complexation of Co²⁺ occurs at pH higher than 6. To prove experimentally this sequential
367 binding behavior, we carried out a pH-spectrophotometric titration of a sample containing equimolar
368 amounts of Cu²⁺, Ni²⁺ and Co²⁺, and 3 equivalents of AcryMet. Starting from pH 2.8 we increased the pH
369 by addition of a solution of KOH until pH 8.8. The absorption spectrum in the visible range was collected
370 at intervals of pH of ca. 0.3 units. The spectral dataset is reported in Figure 7. At pH 2.8 the maximum of

371 absorption is 715 nm, and the intensity of this band increases until pH 4.5. This absorption maximum
372 corresponds to that of $[\text{CuL}]^+$ (see above), and the increase of absorbance follows the increase of $[\text{CuL}]^+$
373 formation as expected from the Cu^{2+} distribution diagram. This intense band shifts at pH higher than 5 to
374 ca. 650 nm as a consequence of the formation of $[\text{Cu}(\text{LH}_-)]$ and $[\text{CuL}_2]$.

375 Interestingly, the absorbance at ca. 900 nm remains low until pH 4.1 and it increases at higher pH
376 values. The absorption of the Cu^{2+} species at this wavelength tends to decrease above pH 4.5, and therefore
377 the increase of intensity at ca. 900 nm is clearly ascribed at the formation of $[\text{NiL}]^+$ which in fact occurs
378 at $\text{pH} > 4$. The low molar absorption of Co^{2+} species did not allow to detect their formation as well defined
379 bands. However, the shoulder at ca. 500-550 nm increases markedly above pH 6, and this change cannot
380 be associated to either Cu^{2+} or Ni^{2+} species which provide a limited spectral change at those wavelengths.
381 Rather, the formation of $[\text{CoL}]^+$ which occurs at $\text{pH} > 6$ explains this quite significant change in the
382 absorption of the shoulder. Overall, these results demonstrate experimentally a sequential complexation
383 of these divalent metal ions in aqueous solution by AcryMet.

384



385

386 **Fig. 7.** UV-visible absorption spectra of the system $\text{Cu}^{2+} / \text{Ni}^{2+} / \text{Co}^{2+} / \text{AcryMet (HL)}$ at different pH values in
387 aqueous solution. $\text{Cu:Ni:Co:L} = 1:1:1:3$, $C_{\text{Cu,Ni,Co}} = 0.00183 \text{ M}$, $I = 0.1 \text{ M KCl}$, $T = 298.2 \text{ K}$, $d = 1 \text{ cm}$, $\text{pH range} =$

388

2.8-8.5.

389 Finally, we have used out equilibrium data to simulate what is the capacity of our ligand to bind metal
390 ions Cu(II), Ni(II) and Co(II) at $1.6 \cdot 10^{-3}$ M (1 ppm) concentration each, and a ligand/total metal content
391 = 1.25:1. The results are reported in Figure S12. Free (uncomplexed) copper at pH 2.5 amounts to 40 %,
392 and total complexation is obtained at pH ca. 4.5. Moreover, Ni(II) results almost entirely complexed at
393 pH 7. Co(II) is the only cation for which no complete complexation is obtained in the pH range 2.5-8.
394 However, at pH 8 less than 50 % total Co(II) is in its free form. Overall these data demonstrate that, at
395 least for Cu(II) and Ni(II), the sequestration capacity of the ligand toward the metal ions is relevant also
396 in trace levels.

397 3.5 Metal Ions Adsorption

398 The kinetic experimental curves, C_t (mg/L) versus t (min) for Cu(II), Co(II) and Ni (II), are shown in Fig.
399 8. The removal of metal due to adsorption is high during the first 180 min for all metal ions. Then, it
400 gradually decreases until equilibrium at 300 min. The rapid adsorption of metal ions in the first three
401 hours may be favored by the presence of several binding sites of the Acry-Met ligand which are available
402 for the adsorption of these intermediate (hard-soft) cations. With the progress of the experiment, the
403 concentration of metals and binding sites decrease, causing a slowdown of the adsorption.

404 The pseudo-first-order kinetics model by Lagergren model [eq. 3] was used to describe the adsorption
405 kinetics of Cu(II), Co(II) and Ni(II) ions onto the Acry-Met film:

$$406 \quad q_t = q_e (1 - esp^{k_1 t}) \quad (3)$$

407

408 where q_t and q_e (mg g^{-1}) are the amounts of metal adsorbed at time t and at the equilibrium time,
409 respectively, and k_1 (min^{-1}) is the pseudo-first-order model rate constant.

410

411

412 Kinetic parameters of pseudo-first-order, obtained from the plot of experimental curves (Fig. 8), and the
413 results are presented in Table 3

414 **Table 3.** Kinetic parameters of metal sorption onto the Acry-Met.

Metal	q_e (mg/g)	R_{ads} (%)	<i>Pseudo first order model</i>	
			$k_l \times 10^{-3}$ (min ⁻¹)	R^2
Cu(II)	1.57	63	0.07	96
Co(II)	1.14	46	0.05	99
Ni(II)	0.89	36	0.04	97

415
416 Overall, data in Table 3 show a membrane loaded with Acry-Met, even if the latter is just dispersed in
417 TEOSs with no precise control of the reticulation of the material, is efficient to remove these two metal
418 ions from the bulk of the solution. Cu(II) presents the highest amount of metal absorbed q_e parameter and
419 the highest rate constant among the three examined. This is perhaps expected for the metal which is
420 highest in the Irving-William series, and which present high rates of substitution of coordinated ligands.

421

422 **4. Conclusions**

423 The removal of toxic metal ions from wastewater is one of the most important targets of current
424 research due to the harmful effects that this kind of pollutants can produce on the human health and
425 environment. In this work the synthesis and characterization of the AcryMet ligand has been presented,
426 together with the X-ray characterization of a 1:2 Cu²⁺/ligand complex. The study of the speciation of
427 divalent cations and especially Cu²⁺, Ni²⁺ and Co²⁺ / AcryMet systems is reported and discussed.

428 AcryMet is the monomeric analogue of the building blocks that constitute polyamidoaminic materials
429 that we have previously reported in the literature. Here we have demonstrated that the complexation of
430 divalent cations basically pivots around the presence of the secondary amine. Further interactions with
431 the metal ions, as clearly proved for Cu²⁺, occurs by the involvement of the carboxylate function and, at

432 high pH, of the deprotonated amidic nitrogen. Perhaps most importantly, the presence of the deprotonated
433 acidic group close to secondary amine in AcryMet is responsible of the sequential binding behavior of
434 the ligand. Actually, the ligand forms complexes with Cu^{2+} at pH around 2.5-3 by virtue of the presence
435 of this deprotonated carboxylic function. The complexation of Ni^{2+} starts at $\text{pH} > 3.5$ while that of Co^{2+}
436 only occurs at $\text{pH} > 6$.

437 Transferred at the polymer level, these observations suggest that metal ions may interact at the
438 secondary amino groups also with the corresponding PAA. Additional interactions may occur at the
439 deprotonated amidic nitrogen where the flexibility of the polymeric chains and the pH conditions allow
440 it. Also, depending on the monomeric components used to prepare the polymers, we may observe the
441 interaction of metal ions with carboxylate functions close to secondary amines. We hope in the future not
442 only to transfer the metal coordination capability of AcryMet into a polymeric material, but also to impart
443 the same material with sequential-binding complexation capabilities. The latter will be extremely
444 interesting in the perspective of sequestration and release of divalent metal cations from water samples,
445 which may occur with sequential selection by simply changing the pH of the solution.

446

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452

453 **References**

454 Alberti, G., Mussi, M., Quattrini, F., Pesavento, M., & Biesuz, R. (2018). Metal complexation capacity
455 of Antarctic lacustrine sediments. *Chemosphere*, 196, 402-408.
456 <https://doi.org/10.1016/j.chemosphere.2017.12.188>

457 Alderighi, L., Gans, P., Ienco, A., Peters, D., Sabatini, A., Vacca, A., 1999. Hyperquad simulation and
458 speciation (HySS): a utility program for the investigation of equilibria involving soluble and partially
459 soluble species. *Coordination chemistry reviews*, 184(1), 311-318. <https://doi.org/10.1016/S0010->
460 [8545\(98\)00260-4](https://doi.org/10.1016/S0010-8545(98)00260-4)

461 Bailey, S. E., Olin, T. J., Bricka, R. M., Adrian, D. D., 1999. A review of potentially low-cost sorbents
462 for heavy metals. *Water research*, 33(11), 2469-2479. [https://doi.org/10.1016/S0043-1354\(98\)00475-8](https://doi.org/10.1016/S0043-1354(98)00475-8)

463 Barbucci, R., Barone, V., Ferruti, P., Delfini, M., 1980. Macro-inorganics. Part 3. Chelation of copper
464 (II) ion with some polymers having a poly (amido-amine) structure and their non-macromolecular models.
465 *Journal of the Chemical Society, Dalton Transactions*, (2), 253-256.
466 <https://doi.org/10.1039/DT98000000253>

467 Bergamonti, L., Graiff, C., Tegoni, M., Predieri, G., Bellot-Gurlet, L., Lottici, P. P., 2017a. Raman and
468 NMR kinetics study of the formation of amidoamines containing N-hydroxyethyl groups and
469 investigations on their Cu (II) complexes in water. *Spectrochimica Acta Part A: Molecular and*
470 *Biomolecular Spectroscopy*, 171, 515-524. <https://doi.org/10.1016/j.saa.2016.07.041>

471 Bergamonti, L., Berzolla, A., Chiappini, E., Feci, E., Maistrello, L., Palanti, S., ... Vaccari, G., 2017b.
472 Polyamidoamines (PAAs) functionalized with siloxanes as wood preservatives against fungi and insects.
473 *Holzforschung*, 71(1), 65-75. <https://doi.org/10.1515/hf-2016-0010>

474 Bergamonti, L., Graiff, C., Tegoni, M., Predieri, G., Elviri, L., Palanti, S., ... Lottici, P. P., 2019. Facile
475 preparation of functionalized poly (amidoamine) s with biocidal activity on wood substrates. *European*
476 *Polymer Journal*, 116, 232-241. <https://doi.org/10.1016/j.eurpolymj.2019.04.027>

477 Betiha, M. A., Moustafa, Y. M., El-Shahat, M. F., & Rafik, E. (2020). Polyvinylpyrrolidone-
478 Aminopropyl-SBA-15 schiff Base hybrid for efficient removal of divalent heavy metal cations from
479 wastewater. *Journal of Hazardous Materials*, 122675. <https://doi.org/10.1016/j.jhazmat.2020.122675>

480 Billo, E. J., 1974. Copper (II) chromosomes and the rule of average environment. *Inorganic and Nuclear*
481 *Chemistry Letters*, 10(8), 613-617. [https://doi.org/10.1016/0020-1650\(74\)80002-4](https://doi.org/10.1016/0020-1650(74)80002-4)

482 Bo, S., Luo, J., An, Q., Xiao, Z., Wang, H., Cai, W., ... Li, Z., 2020. Efficiently selective adsorption of
483 Pb (II) with functionalized alginate-based adsorbent in batch/column systems: Mechanism and
484 application simulation. *Journal of Cleaner Production*, 250, 119585.
485 <https://doi.org/10.1016/j.jclepro.2019.119585>

486 Brahmi, K., Bouguerra, W., Harbi, S., Elaloui, E., Loungou, M., & Hamrouni, B. (2018). Treatment of
487 heavy metal polluted industrial wastewater by a new water treatment process: ballasted
488 electroflocculation. *Journal of Hazardous Materials*, 344, 968-980.
489 <https://doi.org/10.1016/j.jhazmat.2017.11.051>

490 Bruker, 2015. APEX3 and SAINT. Bruker AXS Inc., Madison, Wisconsin, USA

491 Byun, I. S., Han, K. S., Ga, H. R., 2017. Process for the racemization of α -amino acids. U.S. Patent No.
492 9,598,353. Washington, DC: U.S. Patent and Trademark Office.

493 Casolaro, M., Bignotti, F., Sartore, L., Penco, M., Ferruti, P., 1998. Stability of metal complexes with
494 basic polymers in the free, cross-linked and silica-grafted forms. *Current Trends in Polymer Science.*, 3,
495 173-182.

496 Chandrarekha, M., Srinivasan, N., Krishnakumar, R. V., 2015. Redetermined crystal structure of N-(β -
497 carboxyethyl)- α -isoleucine. *Acta Crystallographica Section E: Crystallographic Communications*, 71(9),
498 o665-o666. <https://doi.org/10.1107/S2056989015014498>

499 Esshaimi, M., Ouazzani, N., Avila, M., Perez, G., Valiente, M., & Mandi, L. (2012). Heavy metal
500 contamination of soils and water resources Kettara abandoned mine. *American Journal of Environmental*
501 *Sciences*, 8(3), 253-261. <https://doi.org/10.3844/ajessp.2012.253.261>

502 Duarte A.S.R., Amorim Da Costa A.M, Amado A.M., 2005. On the conformation of neat acrylamide
503 dimers - a study by ab initio calculations and vibrational spectroscopy, *Journal of Molecular Structure:*
504 *THEOCHEM* 723, 63–68, <http://dx.doi.org/10.1016/j.theochem.2005.02.008>.

505 Farrugia, L. J., 2012. WinGX and ORTEP for Windows: an update. *Journal of Applied Crystallography*,
506 45(4), 849-854. <https://doi.org/10.1107/S0021889812029111>

507 Febrianto, J., Kosasih, A. N., Sunarso, J., Ju, Y. H., Indraswati, N., Ismadji, S., 2009. Equilibrium and
508 kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies. *Journal of*
509 *Hazardous Materials*, 162(2-3), 616-645. <https://doi.org/10.1016/j.jhazmat.2008.06.042>

510 Ferruti P., 2013. Poly(amidoamine)s: Past, Present, and Perspectives *Journal of Polymer Science, Part*
511 *A: Polymer Chemistry*, 51, 2319–2353, <https://doi.org/10.1002/pola.26632>

512 Ferruti, P., Danzo, N., Oliva, L., Barbucci, R., Barone, V., 1981. Macro-inorganics. Part 6. Protonation
513 and complex formation of a new series of polymers whose repeating units behave independently. *Journal*
514 *of the Chemical Society, Dalton Transactions*, (2), 539-542. <https://doi.org/10.1039/DT9810000539>

515 Ferruti, P., Marchisio, M. A., Duncan, R., 2002. Poly (amido-amine) s: Biomedical Applications.
516 *Macromolecular Rapid Communications*, 23(5-6), 332-355. [http://dx.doi.org/10.1002/1521-](http://dx.doi.org/10.1002/1521-3927(20020401)23:5/6b332::AID-MARC332N3.0.CO;2-I)
517 [3927\(20020401\)23:5/6b332::AID-MARC332N3.0.CO;2-I](http://dx.doi.org/10.1002/1521-3927(20020401)23:5/6b332::AID-MARC332N3.0.CO;2-I)

518 Ferruti, P., Mauro, N., Falciola, L., Pifferi, V., Bartoli, C., Gazzarri, M., ... Ranucci, E., 2014. Amphoteric,
519 Prevaingly Cationic l-A rginine Polymers of Poly (amidoamino acid) Structure: Synthesis, Acid/B ase
520 Properties and Preliminary Cytocompatibility and Cell-P ermeating Characterizations. *Macromolecular*
521 *bioscience*, 14(3), 390-400. <https://doi.org/10.1002/mabi.201300387>

522 Ferruti, P., Oliva, L., Barbucci, R., Tanzi, M. C., 1980. Macro inorganics V. Basicity and complexing
523 ability of a new class of poly (amido-amines) with tertiary amino groups present both in the main chain
524 and as side substituent. *Inorganica Chimica Acta*, 41, 25-29. [https://doi.org/10.1016/S0020-](https://doi.org/10.1016/S0020-1693(00)88426-1)
525 1693(00)88426-1

526 Ferruti, P., Ranucci, E., Manfredi, A., Mauro, N., Ferrari, E., Bruni, R., ... Rossi, M., 2012. L-lysine and
527 EDTA polymer mimics as resins for the quantitative and reversible removal of heavy metal ion water
528 pollutants. *Journal of Polymer Science Part A: Polymer Chemistry*, 50(24), 5000-5010.
529 <https://doi.org/10.1002/pola.26330>

530 Fu, F., Wang, Q., 2011. Removal of heavy metal ions from wastewaters: a review. *Journal of*
531 *environmental management*, 92(3), 407-418. <https://doi.org/10.1016/j.jenvman.2010.11.011>

532 Gans, P., Sabatini, A., Vacca, A., 1996. Investigation of equilibria in solution. Determination of
533 equilibrium constants with the HYPERQUAD suite of programs. *Talanta-Oxford*, 43(10), 1739-1754.
534 [https://doi.org/10.1016/0039-9140\(96\)01958-3](https://doi.org/10.1016/0039-9140(96)01958-3)

535 Gillard, R. D., P. O'Brien, 1978. The isomers of α -amino-acids with copper (II). Part 4. Catalysis of the
536 racemization of optically active alanine by copper (II) and pyruvate in alkaline solution. *Journal of the*
537 *Chemical Society, Dalton Transactions* 11, 1444-1447. <https://doi.org/10.1039/DT9780001444>

538 Girardi, F., Bergamonti, L., Isca, C., Predieri, G., Graiff, C., Lottici, P. P., ... Di Maggio, R., 2017.
539 Chemical–physical characterization of ancient paper with functionalized polyamidoamines (PAAs).
540 *Cellulose*, 24(2), 1057-1068. <https://doi.org/10.1007/s10570-016-1159-8>

541 Hurd, R. E., 1990. Gradient-enhanced spectroscopy. *J. Magn. Reson.*, 87, 422-428.
542 [https://doi.org/10.1016/0022-2364\(90\)90021-Z](https://doi.org/10.1016/0022-2364(90)90021-Z)

543 Isca, C., D'Avorgna, S., Graiff, C., Montanari, M., Ugozzoli, F., Predieri, G., 2016. Paper preservation
544 with polyamidoamines: a preliminary study. *Cellulose*, 23(2), 1415-1432.
545 <http://dx.doi.org/10.1007/s10570-016-0880-7>

546 Joseph, L., Jun, B. M., Flora, J. R., Park, C. M., & Yoon, Y., 2019. Removal of heavy metals from water
547 sources in the developing world using low-cost materials: A review. *Chemosphere*, 229, 142-159.
548 <https://doi.org/10.1016/j.chemosphere.2019.04.198>

549 Ju, S., Eom, Y., Kim, S. Y., Hwang, S. Y., Hwang, D. S., Oh, D. X., Park, J., 2019. Lysine-cyclodipeptide-
550 based polyamidoamine microparticles: Balance between the efficiency of copper ion removal and
551 degradation in water. *Chemical Engineering Journal*, 123493. <https://doi.org/10.1016/j.cej.2019.123493>

552 Lachowicz, J. I., Crespo-Alonso, M., Caltagirone, C., Alberti, G., Biesuz, R., Orton, J. O., & Nurchi, V.
553 M. (2018). Salicylamide derivatives for iron and aluminium sequestration. From synthesis to
554 complexation studies. *Journal of Trace Elements in Medicine and Biology*, 50, 580-588.
555 <https://doi.org/10.1016/j.jtemb.2018.04.010>

556 Lakherwal, D., 2014. Adsorption of heavy metals: a review. *International journal of environmental*
557 *research and development*, 4(1), 41-48. <http://www.ripublication.com/ijerd.htm>

558 Lee, K. M., Lai, C. W., Ngai, K. S., Juan, J. C., 2016. Recent developments of zinc oxide based
559 photocatalyst in water treatment technology: a review. *Water research*, 88, 428-448.
560 <https://doi.org/10.1016/j.watres.2015.09.045>

561 Levitt, M. H.; Freeman, R.; Frenkiel, T., 1982. Broadband heteronuclear decoupling. *Journal of Magnetic*
562 *Resonance (1969)*, 47, 328-330. [https://doi.org/10.1016/0022-2364\(82\)90124-X](https://doi.org/10.1016/0022-2364(82)90124-X)

563 Li, T., Zhang, W., Zhai, S., Gao, G., Ding, J., Zhang, W., ... Lv, L., 2018. Efficient removal of nickel (II)
564 from high salinity wastewater by a novel PAA/ZIF-8/PVDF hybrid ultrafiltration membrane. *Water*
565 *research*, 143, 87-98. <https://doi.org/10.1016/j.watres.2018.06.031>

566 Lim, M. C., Chen, W., Ali, H. M., 1994. Crystal and molecular structures of bis (N-propionamido-
567 glycinato) copper (II) hydrate, bis (N, N-dipropionamido-glycinato) copper (II) dihydrate and bis (N, N-
568 dipropionamido-glycinato)- μ -carboxylato-dicopper (II) perchlorate hydrate. *Transition Metal Chemistry*,
569 *19(4)*, 409-412. <https://doi.org/10.1007/BF00139316>

570 Malik, L. A., Bashir, A., Qureashi, A., & Pandith, A. H., 2019. Detection and removal of heavy metal
571 ions: a review. *Environmental Chemistry Letters*, 1-27. <https://doi.org/10.1007/s10311-019-00891-z>

572 Manfredi, A., Mauro, N., Terenzi, A., Alongi, J., Lazzari, F., Ganazzoli, F., ... Ferruti, P., 2017. Self-
573 ordering secondary structure of D-and L-arginine-derived polyamidoamino acids. *ACS Macro Letters*,
574 6(9), 987-991. <https://doi.org/10.1021/acsmacrolett.7b00492>

575 Manfredi, A., Ranucci, E., Morandi, S., Mussini, P. R., Ferruti, P., 2013. Fast and quantitative manganese
576 sorption by polyamidoamine resins. *Journal of Polymer Science Part A: Polymer Chemistry*, 51(4), 769-
577 773. <https://doi.org/10.1002/pola.26462>

578 Marcovecchio, J.E., Botté, S.E., Freije, R. H., 2007. Heavy metals, major metals, trace elements.
579 *Handbook of water analysis*, 2, 275-311.

580 Mathews, I.I., Manohar H., 1991) A novel amino acid racemization in vitamin b6-amino acid schiff base
581 copper complexes: crystal structures of aquo (5'-phosphopyridoxylidene-dl-tyrosinato) copper (II) 3.5
582 H₂O and aquo (5'-phosphopyridoxylidene-dl-phenylalaninato) copper (II) 2.5 H₂O. *Polyhedron* 10.18
583 2163-2169. [https://doi.org/10.1016/S0277-5387\(00\)86136-7](https://doi.org/10.1016/S0277-5387(00)86136-7)

584 Nasir A.M., Goh P.S., Abdullah M.S., Cheer N.B., Ismail A.F., 2019. Adsorptive nanocomposite
585 membranes for heavy metal remediation: Recent progresses and challenges. *Chemosphere* 232, 96e112.
586 <https://doi.org/10.1016/j.chemosphere.2019.05.174>

587 Nehls, I., Hanebeck, O., Becker, R., Emmerling, F., 2013. N-(β-Carboxyethyl)-α-isoleucine. *Acta*
588 *Crystallographica Section E: Structure Reports Online*, 69(2), o172-o173.
589 <https://doi.org/10.1107/S160053681205146X>

590 Pan, B., Zhang, Q., Du, W., Zhang, W., Pan, B., Zhang, Q., ... & Zhang, Q., 2007. Selective heavy metals
591 removal from waters by amorphous zirconium phosphate: Behavior and mechanism. *Water*
592 *Research*, 41(14), 3103-3111. <https://doi.org/10.1016/j.watres.2007.03.004>

593 Prenesti, E., Daniele, P. G., Prencipe, M., Ostacoli, G., 1999. Spectrum–structure correlation for visible
594 absorption spectra of copper (II) complexes in aqueous solution. *Polyhedron*, 18(25), 3233-3241.
595 [https://doi.org/10.1016/S0277-5387\(99\)00279-X](https://doi.org/10.1016/S0277-5387(99)00279-X)

596 Quaretti, M., Porchia, M., Tisato, F., Trapananti, A., Aquilanti, G., Damjanović, M., ... Tegoni, M., 2018.
597 Thermodynamic stability and structure in aqueous solution of the [Cu (PTA)₄]⁺ complex (PTA=
598 aminophosphine-1, 3, 5-triaza-7-phosphaadamantane). *Journal of inorganic biochemistry*, 188, 50-61.
599 <https://doi.org/10.1016/j.jinorgbio.2018.08.008>

600 Rajalakshmi, V., Vijayaraghavan, V. R., Varghese, B., Raghavan, A., 2008. Novel Michael addition
601 products of bis (amino acidato) metal (II) complexes: Synthesis, characterization, dye degradation, and
602 oxidation properties. *Inorganic chemistry*, 47(13), 5821-5830. <https://doi.org/10.1021/ic800086y>

603 Rezania, S., Taib, S. M., Din, M. F. M., Dahalan, F. A., Kamyab, H., 2016. Comprehensive review on
604 phytotechnology: heavy metals removal by diverse aquatic plants species from wastewater. *Journal of*
605 *Hazardous Materials*, 318, 587-599. <https://doi.org/10.1016/j.jhazmat.2016.07.053>

606 Rinaldi, P. L.; Keifer, P. A., 1994. The utility of pulsed-field gradient HMBC for organic structure
607 determination. *Journal of Magnetic Resonance, Series A*, 108, 259-262.
608 <https://doi.org/10.1006/jmra.1994.1121>

609 Sheldrick, G. M., 2015. Crystal structure refinement with SHELXL. *Acta Crystallographica Section C:*
610 *Structural Chemistry*, 71(1), 3-8. <https://doi.org/10.1107/S2053229614024218>

611 Smith, R. M.; Martell, A. E.; Motekaitis, R. J. NIST Critically Selected Stability Constants of Metal
612 Complexes Database. NIST: Gaithersburg, MD, USA 2007

613 Sigel, H., Martin, R. B., 1982. Coordinating properties of the amide bond. Stability and structure of metal
614 ion complexes of peptides and related ligands. *Chemical Reviews*, 82(4), 385-426.
615 <https://doi.org/10.1021/cr00050a003>

616 Tahir, M.B., Kiran, H. Iqbal, T., 2019. The detoxification of heavy metals from aqueous environment
617 using nano-photocatalysis approach: a review. *Environmental Science and Pollution Research* 26, 10515.
618 <https://doi.org/10.1007/s11356-019-04547-x>

619 Tarazona-Vasquez, F., Balbuena, P. B., 2005. Complexation of Cu (II) ions with the lowest generation
620 poly (amido-amine)-OH dendrimers: a molecular simulation study. *The Journal of Physical Chemistry B*,
621 109(25), 12480-12490. <http://dx.doi.org/10.1021/jp051469p>.

622 Uddin, M. K., 2017. A review on the adsorption of heavy metals by clay minerals, with special focus on
623 the past decade. *Chemical Engineering Journal*, 308, 438-462. <https://doi.org/10.1016/j.cej.2016.09.029>

624 Vardhan, K. H., Kumar, P. S., and Panda, R. C., 2019. A review on heavy metal pollution, toxicity and
625 remedial measures: Current trends and future perspectives. *Journal of Molecular Liquids*, 290, 111197.
626 <https://doi.org/10.1016/j.molliq.2019.111197>

627 Vareda, J. P., Valente, A. J., and Durães, L., 2019. Assessment of heavy metal pollution from
628 anthropogenic activities and remediation strategies: A review. *Journal of environmental*
629 *management*, 246, 101-118. <https://doi.org/10.1016/j.jenvman.2019.05.126>

630 Wadhawan, S., Jain, A., Nayyar, J., Mehta, S. K., 2020. Role of nanomaterials as adsorbents in heavy
631 metal ion removal from waste water: A review. *Journal of Water Process Engineering*, 33, 101038.
632 <https://doi.org/10.1016/j.jwpe.2019.101038>

633 Wang, L., Luo, Y., Li, H., Yu, D., Wang, Y., Wang, W., Wu, M., 2020. Preparation and selective
634 adsorption of surface-imprinted microspheres based on hyperbranched polyamide–functionalized sodium
635 alginate for the removal of Sb (III). *Colloids and Surfaces A: Physicochemical and Engineering Aspects*,
636 585, 124106. <https://doi.org/10.1016/j.colsurfa.2019.124106>

637 Weinstein, G. N., M. J. O'connor, R. H. Holm., 1970) Preparation, properties, and racemization kinetics
638 of copper (II)-Schiff base-amino acid complexes related to vitamin B6 catalysis. *Inorganic Chemistry*,
639 9.9 2104-2112. <https://doi.org/10.1021/ic50091a029>

640 Wilker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W., 1993. Gradient selection in inverse heteronuclear
641 correlation spectroscopy. *Magnetic Resonance in Chemistry*, 31, 287-292.
642 <https://doi.org/10.1002/mrc.1260310315>

643 Xiaoli, C., Shimaoka, T., Xianyan, C., Qiang, G., & Youcai, Z. (2007). Characteristics and mobility of
644 heavy metals in an MSW landfill: Implications in risk assessment and reclamation. *Journal of hazardous*
645 *materials*, 144(1-2), 485-491. <https://doi.org/10.1016/j.jhazmat.2006.10.056>

646 Xu, Y., Zhao, D., 2005. Removal of copper from contaminated soil by use of poly (amidoamine)
647 dendrimers. *Environmental science technology*, 39(7), 2369-2375. <http://dx.doi.org/10.1021/es040380e>

648 Zheng, X., Liu, T., Guo, M., Li, D., Gou, N., Cao, X., ... & Pan, B., 2020. Impact of heavy metals on the
649 formation and properties of solvable microbiological products released from activated sludge in biological
650 wastewater treatment. *Water Research*, 115895. <https://doi.org/10.1016/j.watres.2020.115895>

651 Zhou, G., Luo, J., Liu, C., Chu, L., & Crittenden, J. (2018). Efficient heavy metal removal from industrial
652 melting effluent using fixed-bed process based on porous hydrogel adsorbents. *Water research*, 131, 246-
653 254. <https://doi.org/10.1016/j.watres.2017.12.067>

654 Zintchenko, A., van der Aa, L. J., Engbersen, J. F., 2011. Improved synthesis strategy of poly
655 (amidoamine)s for biomedical applications: catalysis by “green” biocompatible earth alkaline metal salts.
656 *Macromolecular rapid communications*, 32(3), 321-325. <http://dx.doi.org/10.1002/marc.201000545>

657