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Research paper

Penta-glycine copper(II) complexes in slightly alkaline solutions

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ABSTRACT

The kinetics of the reaction of Cu^{II}(GGG), Cu^{II}(GGGGG) and Cu^{II}(GGGGS), where G = glycine and S = sarcosine, with 4-nitrophenyl-acetate were measured. The results point out that at pH 9.0 hydroxide is an axial, or equatorial ligand to a significant amount of these complexes. The source of the different mechanisms of reaction of the analogous nickel complexes is attributed to the axial water ligand of the copper complexes. The biological implications of axial hydroxide binding to copper peptides is discussed.

1. Introduction

Copper complexes with proteins [1–8] (These are only some recent reviews and not a full literature survey) and peptides [9–17] (These are only some recent reviews and not a full literature survey) are of major biological importance. Therefore the study of the nature of copper complexes with a variety of peptides and proteins, many of them include potentiometric titrations that point out that the amide nitrogens are gradually deprotonated as the pH is raised, has been performed [9–11,13,14,16–20]. Copper prefers binding to histidine [3,6,7,11,12,14–16,18] and cysteine [5,7,8,11] and to the terminal amine groups of peptides [9,11,14,17,18]. The latter observation is somewhat surprising as the deprotonated amides are better σ donors than the terminal amine, as can be deduced from the shift in the d → d absorption bands of the corresponding complexes [19,21]. The crystal structure of the copper(II) complex with penta-glycine, Cu^{II}(GGGGG) [9], and the spectra of this complex in solution [18] suggest that the copper is bound to the terminal amine even in slightly alkaline solutions, though the deprotonated amides are better σ donors than the terminal amine.

The reaction of nucleophiles with 4-nitrophenyl-acetate, p-NPA, results in the binding of acetate to the nucleophile [22] (and references therein). Thus the reactions of p-NPA with substrates are useful to determine the site of nucleophilicity of a given reagent. Recently the study of the kinetics and products of the nucleophilic reaction of Ni^{II}(GGGGG) with p-NPA pointed out that Ni^{II}(GGGGG) at pH 9.0 is present as an equilibrium of *ca.* 50% of the nickel ions bound to four deprotonated amide bonds and *ca.* 50% to the terminal amine, as the product of the reaction is acetylated GGGGG [22]. It seemed of interest to check, using the same reaction, whether Cu^{II}(GGGGG) behaves similarly and if not why. For comparison also the properties of the complexes Cu^{II}(dimethyl-GGGGG), in which the terminal amine is expected to be a considerably weaker ligand (tertiary amines are known to be poor ligands for high valent transition metal complexes [23–25], including Cu(II) complexes [23]) and Cu^{II}(GGGGS), where S = sarcosine which cannot form a tetra-de-pronated ligand were studied.

2. Experimental

2.1. Materials

4-Nitrophenyl-acetate and all the inorganic materials, A.R. grade, were purchased from Sigma-Aldrich and were used without further purification. Tri- and penta-glycine were purchased from Chem-Impex International. Water was deionized and further purified by passing through a Millipore Milli-Q setup with a final resistivity >10 MΩ cm^{-1}. Dimethyl-GGGGG was synthesized according to Scheme 1.

The Penta-peptide GGGGS was synthesized according to Scheme 2.

The peptides were characterized by ^{1}H and ^{13}C NMR and by ESI-MS. The results are presented in the Supplementary material.

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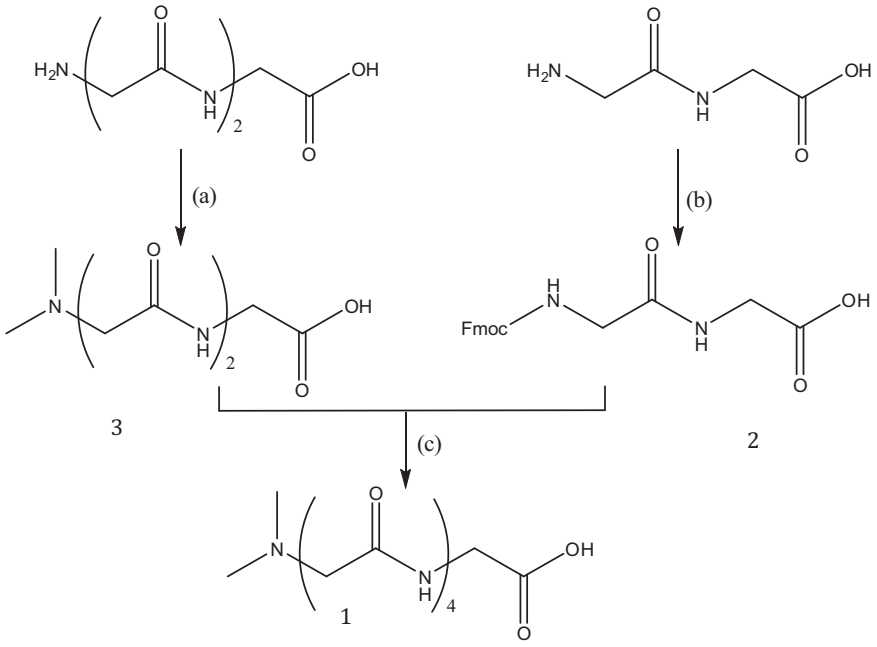
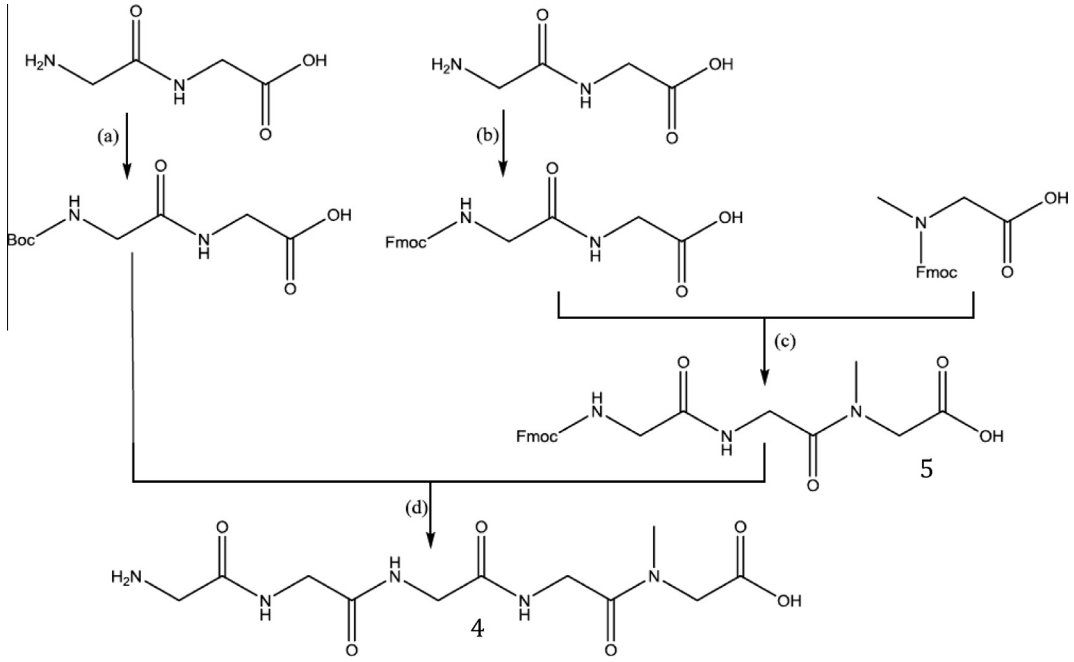


Scheme 1. (a) CH_2O, H_2O, Pd/C 10%, H_2 (4 bar); (b) Fmoc-Cl, Na_2CO_3, dioxane-water, room temperature, 12 h; (c) SPPS on Cl-Trt resin, PyBOP, DIEA (for details see Supporting information).



Scheme 2. (a) $(BOC)_2O$, THF-H_2O, TEA, 12 h; (b) Fmoc-Cl, Na_2CO_3, Dioxane-Water, rt, 12 h; (c) SPPS on Cl-Trt resin, TriPhosgen, collidine; (d) SPPS on Cl-Trt resin, PyBOP, DIEA (for details see Supporting information).

2.2. Spectrophotometric and kinetic measurements

All the spectrophotometric measurements and kinetic studies were carried out using an Agilent 8453 Diode Array spectrophotometer. The reactions were monitored by following the increase in absorbance at 400 nm, which corresponds to the formation of the product para-nitro-phenolate, p-NP. The reactions were carried out under pseudo-first order conditions – peptide or complex in large excess over 4-NPA. The complexes were prepared in the presence of excess $CuSO_4$, so that no free peptide was present. All the kinetics fitted a first order rate law and the rate constants are the average of results obtained from at least two independently prepared samples, for each sample at least three kinetic runs were performed, error limit <10%.

2.3. Solution preparation

The peptide complexes for spectrophotometric measurements were prepared by dissolving $1.5 \cdot 10^{-4}$ mol of the peptide in ~10 ml water. To this solution $1.26 \cdot 10^{-4}$ mol of $CuSO_4$, from a

stock solution of 0.20 M, were added and the volume of the solution was adjusted to 25 ml, yielding a solution containing 5.1 mM Cu^{II}(peptide). The pH of the solutions was adjusted with 0.010 M NaOH. (As GGGGG has a poor solubility it was dissolved in an alkaline solution to which the $CuSO_4$ solution was added very slowly and the pH was adjusted with $HClO_4$.)

2.4. Product analysis

The products of the reaction were analyzed by ESI-MS.

3. Results and discussion

First the reaction of Cu^{II}(GGG) with 4-nitrophenyl-acetate, 4-NPA, was studied as in this complex clearly the central copper cation cannot move and the terminal amine is always bound to the copper cation. The results are presented in Fig. 1. The results at pH 8.0 are analogous to those earlier reported for Ni^{II}(GGG), [22] *i.e.* the observed rate constant is independent on [Cu^{II}(GGG)]. However, the linear dependence of the rate constant on [Cu^{II}(-GGG)] at pH 9.0 is surprising, as clearly the rise of the pH cannot affect the binding of the terminal amine to the copper central cation. To verify this the products of the reaction were analyzed by ESI-MS and indeed no acetylation of the peptide is observed. Thus one has to conclude that the raise in the pH causes a binding of an axial hydroxide to the copper cation, and this hydroxide acts as the nucleophile in the reaction (Scheme 3).

It is of interest to note that the observed rate constant for the reaction at pH 9.0 is only somewhat lower than that reported for the reaction of Ni^{II}(GGGGG) with p-NPA at this pH [22]. This result suggests that the hydroxide bound to the Cu^{II}(GGG) is a nucleophile with similar properties to the free terminal amine of Ni^{II}(-GGGGG) at this pH. It should be noted that there is no solid evidence for the site of binding of the hydroxide and it might replace the carboxylate that might then be the axial ligand, see discussion below.

Next the reaction of Cu^{II}(GGGGG) with p-NPA at pH 9.0 was studied, the results are presented in Fig. 2. It should be noted that at pH 8.0 a precipitate is formed in the presence of Cu^{II}(GGGGG) even in the absence of p-NPA. (The precipitate was shown to contain both Cu and GGGGG.)

The observed rate constant of the reaction is similar to that observed for Cu^{II}(GGG) at this pH but also to that reported for Ni^{II}(-GGGGG) at this pH and to that of GGGGG [22]. In order of determining the mechanism of reaction the products of reaction were determined by ESI-MS: no acetylated peptide was observed pointing out that also in this system the reaction of Cu^{II}(GGGGG)(OH) is observed, the results clearly point out that in less than 10% of the complex the terminal amine is not bound to the central copper cation, as 10% of acetylation of the ligand would have been detected by the ESI-MS analysis. The conclusion that hydroxide ions are axially bound at pH 9.0 to Cu^{II}(GGG) and Cu^{II}(GGGGG) differs from the structures suggested in the literature for copper peptides at similar pHs [13,18,19]. However, this result is not totally surprising as it was proposed that *ca.* 11% of the triply deprotonated Cu^{II}(GGGG) are present as $[Cu(H_{-2}G_4(OH)]^{2-}$ where the hydroxide is probably bound in an equatorial position [26], though clearly the results presented herein indicate that for the triply-deprotonated, or tetra-deprotonated, Cu^{II}(GGGGG) a considerably larger fraction is present as $[Cu(H_{-2}G_5(OH)]^{2-}$ or as $[Cu(H_{-3}G_5(OH)]^{3-}$ and not as $[Cu(H_{-3}G_5]^{2-}$, or $[Cu(H_{-4}G_5]^{3-}$, where the hydroxide is probably bound in an axial position. As potentiometric titrations [18] of Cu^{II}(GGGGG) indicate that even at pH 10.0 only $[Cu(H_{-3}G_5]^{2-}$ is nearly the only form of the complex present in solution and at pH 9.0 a mixture of *ca.* 1:1 of $[Cu(H_{-2}G_5]^{-}$ and $[Cu(H_{-3}G_5]^{2-}$ is present [18] our results indicate that the species present in the solution, at least in part, is $[Cu(H_{-2}G_5(OH)]^{2-}$ and not $[Cu(H_{-3}G_5]^{2-}$. Clearly potentiometric titrations cannot differentiate between $[Cu(H_{-2}G_5(OH)]^{2-}$ and not $[Cu(H_{-3}G_5]^{2-}$.

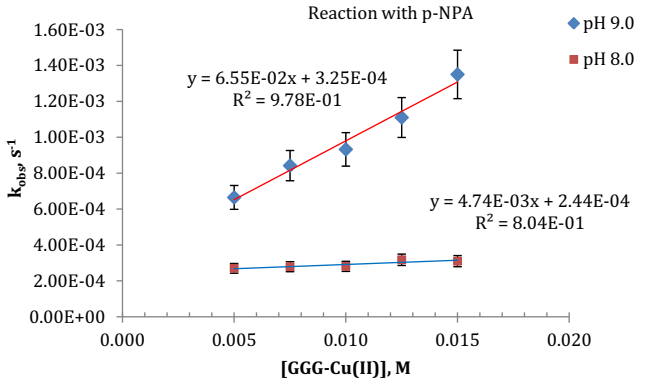


Fig. 1. Dependence of the rate of hydrolysis of p-NPA on [Cu^{II}(GGG)]. [GGG]/[$CuSO_4$] = 1.19, [p-NPA] = 5×10^{-4} M. (The rate constants are the average values of all the measured kinetics ± 10%.)

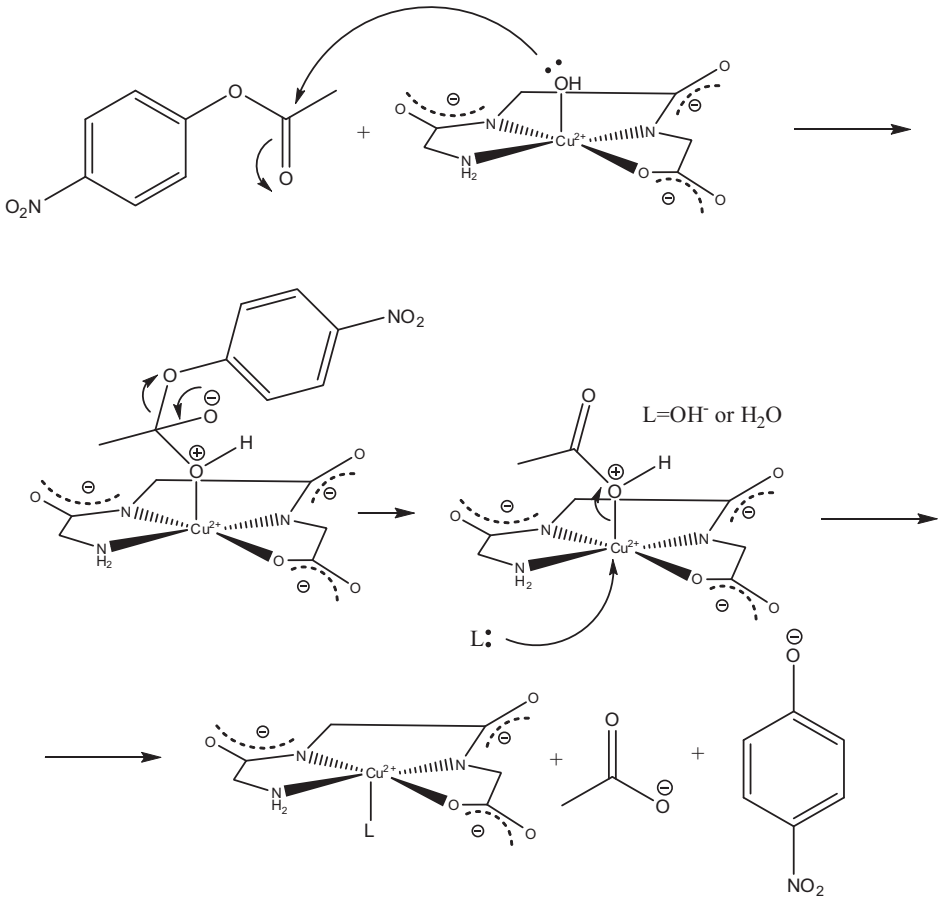
Similar results are obtained, as expected, also for the reaction of Cu^{II}(GGGGS), *i.e.* no acetylation of the peptide is observed. Surprisingly the reaction of Ni^{II}(GGGGS) with p-NPA at pH 9.0 yields the acylated peptide, $CH_3C(O)NH(CH_2C(O)NH)_3(CH_2N(CH_3)\text{-}CH_2COO^-$. This result proves that the terminal amine is not bound to the central Ni^{II} cation though clearly the sarcosine amide cannot be deprotonated.

In an effort to determine the nature of the complexes in alkaline pHs, 10 < pH < 11 the spectra of the solutions were measured. The results are summed up in Table 1.

Clearly UV–Vis. spectra do not prove a structure but they might support the kinetic results. The source of the discrepancy in the experimental results for Cu^{II}(GGG) is not clear. However, it was suggested that axial binding to copper peptides shifts the absorption band to the red [27], this is in accord with our result. Clearly we cannot determine whether the carboxylate or the hydroxide is the axial ligand. The absorption band of Cu^{II}(GGGGG) is between those calculated for $Cu^{II}(H_{-3}G_5)$ and $Cu^{II}(H_{-4}G_5)$, however the kinetic results above suggest $[Cu^{II}(H_{-2}G_5(OH)]^{2-}$. The absorption bands of the alkaline solutions of Cu^{II}(GGGGG) and Cu^{II}(GGGGS) are very similar clearly suggesting that $Cu(H_{-4}$GGGGG) is not formed as $Cu^{II}(H_{-4}$GGGGS) is impossible. The spectrum of $Cu^{II}((\text{-}CH_3)_2$GGGGG) is considerably shifted to the red in comparison with that of Cu^{II}(GGGGG) this might be due to the fact that the terminal di-methylated amine is a considerably weaker σ-donor than the terminal amine [24] or that it was replaced by a hydroxide or water molecule as an equatorial ligand.

4. Concluding remarks

The kinetic results presented clearly point out that already at pH 9.0 the complexes Cu^{II}(GGGGG) and Cu^{II}(GGGGS) are coordinated, probably axially, at least partially by a hydroxide anion. The degree of hydroxide coordination cannot be determined kinetically as the rate constant of the bound hydroxide with p-NPA is unknown. Also Cu^{II}(GGG) at this pH is coordinated at least partially by a hydroxide anion, in this case the coordination site could not be determined. The observation that hydroxo complexes are formed in slightly alkaline solutions is not surprising as the Cu^{II} complexes: $Cu(H_2O)_5^{2+}$ [29–33], $Cu(NH_3)_5^{2+}$ [34], cis- or trans-Cu^{II}(-amino-acid)$_2(H_2O)$ [35], Cu^{II}(Glycylglycine)$(H_2O)_2$ [36], Cu^{II}(Proteins) [37], are all penta-coordinated. Therefore the



Scheme 3. Proposed mechanism of reaction of Cu^{II}(GGG) with p-NPA at pH 9.0.

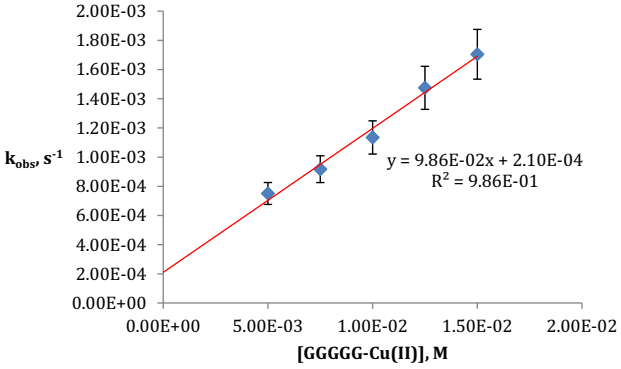


Fig. 2. Dependence of the rate of hydrolysis of p-NPA on [Cu^{II}(GGGGG)]. [GGGGG]/[$CuSO_4$] = 1.19, [p-NPA] = 5×10^{-4} M. (The rate constants are the average values of all the measured kinetics ± 10%.)

hydroxo ligand is not exactly axial as it is probably one of the ligands of a pyramidal complex configuration. (Note added in proof: after the submission of this manuscript the structure of Cu $(H_{-2}GGG)^-$ in neutral aqueous solutions, as studied by EPR; NMR & DFT, was reported to be penta-coordinated square pyramidal with the water being in the axial position [38]. This result corroborates our conclusions.)

The formation of hydroxo complexes at pH 9.0 means that even at physiological pHs some hydroxo complexes are present. This might be of catalytic importance as it is well established that LCu^{II}-OH complexes catalyze, in enzyme like mechanisms, the hydrolysis of: p-nitrophenyl-picolinate [39], p-nitro-phenyl-adamantate [40], p-nitro-phenyldiphenyl-phosphate [41], of amides [42], of phospho-diesters [43] and of DNA [44–46]. Hydrolysis of phenylalanine esters by Cu(II) complexes with poly-L-lysine was also reported.

Finally it should be pointed out that the different behavior of the nickel and copper peptide complexes stems from the electronic configuration of the central cations: The nickel d^8 configuration forms with the strong-field de-protonated peptide ligands planar complexes whereas the copper d^9 Jahn-Teller distorted complexes are penta-coordinated.

Table 1
Spectra of the complexes in alkaline solutions.

Complex	pH	λ_{max} (experimental) nm	λ_{max} (calculated)[a] nm
Cu^{II}(GGG)	10.9	573 (558[b])	556[c]
Cu^{II}(GGGG)	10.9	(514[d])	515[e]
Cu^{II}(GGGGG)	10.4	507 (512[f])	515[e]; 505[g]
Cu^{II}(GGGGS)	10.4	511	515[e]
$Cu^{II}((CH_3)_2GGGGG)$	10.2	533	–

[a] Using Eq. (24) in Ref. [27].
[b] Ref. [28], pH 11.8, suggested ligands: NH_2;N^-; N^-; CO_2^-; OH^-.
[c] Calculated assuming the ligands are: NH_2; N^-; N^-; CO_2^-.
[d] Ref. [25], pH 10.9,suggested ligands: NH_2; N^-; N^-; N^-.
[e] Calculated assuming the ligands are: NH_2; N^-; N^-; N^-.
[f] Ref. [18], suggested ligands: NH_2; N^-; N^-; N^-.
[g] Calculated assuming the ligands are: N^-; N^-; N^-; N^-.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2016.05.055.

References

[1] D. Martinelli, C. Dionisi-Vici, Ann. New York Acad. Sci. 1314 (2014) 55.
[2] J. Telianidis, Y.H. Hung, S. Materia, S.L. Fontaine, Front. Aging Neurosci. 5 (2013) 1.
[3] L. Quintanar, L. Rivillas-Acevedo, R. Grande-Aztatzi, C.Z. Gomez-Castro, T. Acros-Lopez, A. Vela, Coord. Chem. Rev. 257 (2013) 429.
[4] V. Hodgkinson, M.J. Petris, J. Biol. Chem. 287 (2012) 13549.
[5] E. Mocchegiani, L. Costarelli, R. Giacconi, F. Piacenza, A. Basso, M. Malavolta, Ageing Res. Rev. 11 (2012) 297.
[6] F. Zhou, G.L. Millhauser, Coord. Chem. Rev. 256 (2012) 2285.
[7] A.C. Rosenzweig, Nat. Chem. 1 (2009) 684.
[8] I.G. Macreadie, Eur. Biophys. J. 37 (2008) 295.
[9] V. Lillo, J.R. Galan-Mascaros, Dalton Trans. 43 (2014) 9821.
[10] P. Faller, C. Hureau, P. Dorlet, P. Hellwig, Y. Coppel, F. Collin, B. Alies, Coord. Chem. Rev. 256 (2012) 2381.
[11] I. Sovago, C. Kallay, K. Varnagy, Coord. Chem. Rev. 256 (2012) 2225.
[12] G. Arena, D. La Mendola, G. Pappalardo, I. Sovago, E. Rizzarelli, Coord. Chem. Rev. 256 (2012) 2202.
[13] C.R. Dennis, J.C. Swarts, D.W. Margerum, React. Kinet. Mech. Catal. 107 (2012) 27.
[14] P. Faller, C. Hureau, Dalton Trans. (2009) 1080.
[15] L. Pickart, J. Biomater. Sci. Polym. Ed. 19 (2008) 969.
[16] V. Streltsov, Eur. Biophys. J. 37 (2008) 257.
[17] I. Sovago, K. Osz, K. Varnagy, Bioinorg. Chem. Appl. 1 (2003) 123.
[18] K. Varnagy, J. Szabo, I. Sovago, G. Malandrinos, N. Hadjiliadis, D. Sanna, G. Micera, Dalton Trans. (2000) 467.
[19] E. Garriba, G. Micera, J. Chem. Ed. 84 (2007) 832.
[20] M.K. Kim, A.E. Martell, J. Am. Chem. Soc. 88 (1966) 914.
[21] F.P. Bossu, K.L. Chellappa, D.W. Margerum, J. Am. Chem. Soc. 99 (1977) 2195.
[22] Z. Gaisin, G. Gellerman, D. Meyerstein, Eur. J. Inorg. Chem. (2013) 3191.
[23] G. Golub, H. Cohen, P. Paoletti, A. Bencini, L. Messori, I. Bertini, D. Meyerstein, J. Am. Chem. Soc. 117 (1995) 8353.
[24] D. Meyerstein, Coord. Chem. Rev. 185–186 (1999) 141.
[25] T. Clark, M. Hennemann, R. van Eldik, D. Meyerstein, Inorg. Chem. 41 (2002) 2927.
[26] M.P. Youngblood, K.L. Chellappa, C.E. Bannister, D.W. Margerum, Inorg. Chem. 20 (1981) 1743.
[27] H. Sigel, R.B. Martin, Chem. Rev. 82 (1982) 385.
[28] I. Sovago, D. Sanna, A. Dessi, K. Varnagy, G. Micera, J. Inorg. Biochem. 63 (1996) 99.
[29] A. Pasquarello, I. Petri, P.S. Salmon, O. Parisel, R. Car, E. Toth, D.H. Powell, H.E. Fischer, L. Helm, A.E. Merbach, Science 291 (2001) 856.
[30] M. Benfatto, P. D'Angelo, S. Della Longa, N.V. Pavel, Phys. Rev. B 65 (2002). 174205-1-5.
[31] D.T. Brown, M. Amboage, R. Boada, A. Freeman, S. Hayama, S. Diaz-Moreno, RSC Adv. 3 (2013) 17803.
[32] S.R. Qiu, B.C. Wood, P.R. Ehrmann, S.G. Demos, P.E. Miller, K.I. Schaffers, T.I. Suratwala, R.K. Brow, Phys. Chem. Chem. Phys. 17 (2015) 18913.
[33] P. Frank, M. Benfatto, M. Quayyam, B. Hedman, K.O. Hodgson, J. Chem. Phys. 142 (2015). 084310-1-14.
[34] P. Frank, M. Benfatto, B. Hedman, K.O. Hodgson, Inorg. Chem. 47 (2008) 4126.
[35] M.S. Bukharov, V.G. Shtyrlin, A.S. Mukhtarov, G.V. Mamin, S. Stapf, C. Mattea, A. A. Krutikov, A.N. Il'in, N.Y. Serov, Phys. Chem. Chem. Phys. 16 (2014) 9411.
[36] D. Wyrzykowski, D. Zarzeczanska, D. Jacewicz, L. Chmurzynski, J. Therm. Anal. Calorim. 105 (2011) 1043.
[37] K.P. Kepp, Chem. Rev. 112 (2012) 5193.
[38] M.S. Bukharov, V.G. Shtyrlin, G.V. Mamin, S. Stapf, C. Mattea, A.Sh. Mukhtarov, N.Yu. Serov, E.M. Gilyazetdinov, Inorg. Chem. 54 (2015) 9777.
[39] X.M. Kou, G.P. Zhao, Z. Huang, Y.H. Tian, X.G. Meng, X.C. Zeng, Chin. J. Chem. 25 (2007) 772.
[40] W.B. Jeon, K.H. Bae, S.I. Byun, J. Inorg. Biochem. 71 (1988) 163.
[41] S. Bhattacharya, K. Snehaltha, V.P. Kumar, J. Org. Chem. 68 (2003) 2741.
[42] J. Chin, V. Jubian, K. Mrejen, J. Chem. Soc. Chem. Commun. (1990) 1326.
[43] E. Kovari, R. Kramer, J. Am. Chem. Soc. 118 (1996) 12704.
[44] Q. Zhang, Y. Xiang, R. Yang, J. Si, H. Guo, Chin. J. Chem. 29 (2011) 711.
[45] S. Anbu, M. Kandaswamy, Inorg. Chim. Acta 385 (2012) 45.
[46] T. Nozawa, Y. Akimoto, M. Hatano, Die Makromoleculare Chemie 158 (1972) 21.