

Reviewer Report on Ph.D. Thesis of Mgr. Ghazaleh Yassaghi
“Investigation of Copper Complexes Using Mass Spectrometry”

In her Ph.D. thesis, Ghazaleh Yassaghi investigated several types of copper complexes, especially their structures and reactivity in the gas phase. The thesis is divided into three parts. The first part is focused on the complexes with redox-active ligands catechol, maltol and phenanthraquinone. In the second part, negatively charged complexes of copper with one specially designed pincer ligand and several different counter-ions were investigated, and in the third part, the generation and reactivity of copper(III)-oxo complexes with monodentate or bidentate ligands were studied.

The aim of the thesis was to study generation and reactivity of copper(III) complexes with different ligands to better understand properties and reactivity of the terminal Cu-O unit in biological systems.

To this end, several experimental methods were used. The gas-phase complexes were generated by electrospray ionization of appropriately selected solutions of copper salts and respective ligands. Structures of the complexes were studied by collision induced dissociation (CID) mass spectrometry and infrared multiphoton dissociation (IRMPD) spectroscopy. Ion-molecule reactions of several complexes with selected neutral reactants were studied on a triple quadrupole mass spectrometer at different reactant pressures and collision energies. Determination of thermochemical quantities, namely bond dissociation energies or appearance energies, for several complexes was also attempted. The rich experimental part of the thesis is greatly complemented by quantum chemical calculations.

The content of the two subchapters of Chapter 3, “Results and discussion” (reactivity of copper(III)-oxo complexes in the gas-phase and gas-phase study of copper complexes with redox-active ligands) has been already published as two papers in impacted international journals.

I would divide my comments on the thesis into the following two parts.

1. General comments:

- Though I am not a native English speaker, I have to state that the thesis is written in rather simple English. This fact itself would not be a problem if the English was flawless. Unfortunately, it is not, which makes reading it a bit difficult in some cases. The last sentence on page 63 can serve as a typical example (other examples are kindly left to careful readers).
- There are too many typos, inconsistencies and missing important information in Figure captions.

- There are several cases of inconsistent naming. For example, phenanthraquinone is sometimes labeled as PQ and in the very next sentence as **2** (page 47-48). Appearance energies are abbreviated as *AE* on page 48 and as ΔE in adjacent Figure 3.1.11, etc.

- In numerous places, especially in Figure captions, the term *m/z* in connection with equal sign, i.e. $m/z = 562$, was used, which is not recommended by IUPAC. The proper usage is *m/z* 562, i.e. without equal sign.

2. Specific comments:

- Page 24: TSQ 7000 does not have a Daly-type detector (i.e. combination of conversion dynode, scintillator and photomultiplier). Instead, it has just a conversion dynode and an electron multiplier.

- Page 48-49, Figure 3.1.11: It is not clearly stated on which instrument the data presented in this Figure were acquired. Nevertheless, from the context I suppose that it was on the LCQ. If yes, I have a fundamental objection to the methodology of appearance energy measurements on this type of instrument. As it is stated in chapter “2.2.1 LCQ quadrupole ion trap mass spectrometer”, the collision energy scale has to be calibrated. To this end, a set of parent/product ion systems of “known” AE values was used to convert energies from Thermo’s proprietary so-called “normalized collision energies” to the real, physically meaningful appearance energies. The problem is that as the set of “known” AE values a set of calculated AEs was used. In other words, no experimental appearance energies but just theoretical estimates were used to calibrate the energy scale. It means that each “unknown” appearance energy obtained using such calibration is not a real experimental value. In fact, it is again a theoretical estimate, no matter that it was obtained by more expensive and time consuming way using a mass spectrometer instead of a computer code.

From this perspective, any comparison of such, I would say, “quasi experimental” energies with computed theoretical values is a bit meaningless. You will always obtain a good agreement. And if not, there has to be something wrong with some of the calculations involved (insufficiently reliable level of theory used, wrong dissociation channel calculated, etc.)

The reported uncertainties (error bars) of those “quasi experimental” AEs also require a fundamental remark. Each particular theoretical method used for calculation of the reference AE values has its own reliability. Though it is not clear from the thesis, at which level of theory the reference AE values used in Figures 2.3 and 2.4 were calculated, my guess is that one of the popular hybrid DFT functionals (such as B3LYP, B3P86, etc.) in connection with double or triple zeta basis set was employed. If this is the case, I have to stress that even the authors of this method for determination of AEs (E.-L. Zins *et al.*, *J. Mass. Spectrom.* **2010**, 45, 1253–1260) admit that there is a limitation at this point. They refer to the paper of Barylyuk *et al.* [*J. Am. Soc. Mass Spectrom.* **2010**, 21, 172) that shows that the conservative estimated accuracy of ca $\pm 40 \text{ kJ mol}^{-1}$ for the critical energies for the dissociation of benzylpyridinium ions is predicted

with B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d). So when AE values with accuracy of ca ± 40 kJ mol⁻¹ are used for calibration, the uncertainties of the AE values obtained using this calibration can never be in the range of only 0.03 to 0.1 eV (i.e. ca 3-10 kJ/mol) as reported in the thesis. The narrow error bars reported there reflect only a precision with which the normalized collision energy can be set and measured by the LCQ instrument.

- Page 59, Chapter “¹⁸O-labeling experiment”: I think it is self-evident that fragmentation patterns of unlabeled and labeled complex are consistent. So the statement “This result is consistent with the fragmentation pattern of the unlabeled complex” (at the end of page 59) is rather trivial. In my opinion, the only conclusion that can be drawn from this experiment is that only the oxygen atoms from chlorate anion are involved in the fragmentation (i.e. subsequent loss of ClO₂ and H₂O) of the complex and that carbonyl oxygen atoms remain intact. This experiment itself says nothing about the location of the third remaining chlorate oxygen after the loss of ClO₂. The suggested existence of the Cu-O bond is just a chemical intuition supported, of course, by other experiments, theoretical calculations and literature data.

- Page 62: “The hydroxylated ligand then rapidly loses water, ...” I do not think it is “rapid” when the ion at *m/z* 562 in Figure 3.2.2a, i.e. the one that is supposed to lose a water molecule, is still clearly visible in the MS/MS spectrum, i.e. after 30 ms activation.

- Page 65 and Figure 3.2.6: „Minor peaks at *m/z* 563 and 546 can be assigned to ...” In my opinion, the peak at *m/z* 563 can hardly be called “minor” as it is apparently the most abundant ion in the spectrum.

- Page 69, Table 3.2.1: I am able to tolerate using term BDE (bond dissociation energy) in case of bidentate acetate ligand, however, using this term in case of elimination of acetic acid and formaldehyde is, in my opinion, inappropriate. To which bond is the dissociation energy referred to in these particular cases?

- Pages 91-92, Figure 3.3.8: Description of the reaction with H₂O is rather clumsy and misleading. The sentence "It can be observed as a loss of the oxygen atom followed by addition of another water molecule" implies that there is a loss of oxygen atom taking place. However, as far as I understand, the ion [(MeCN)CuO]⁺ attaches H₂O and then a molecule of H₂O₂ is eliminated. It leads to [(MeCN)Cu]⁺ ion, which formally is one oxygen atom less than parent ion, but there is no direct loss of oxygen atom from [(MeCN)CuO]⁺ ion going on, as one could deduce from the above mentioned sentence and red arrow in Figure 3.3.8. Or is there really a loss of O atom?

In conclusion, I would like to emphasize that in spite of my criticism regarding some issues, the submitted thesis represents an extensive set of new experimental and theoretical data that brings new and important information about structure and chemistry of the gas-phase copper complexes that may help to better understand a role of copper oxide moiety in biological systems. Thus the aims and objectives of the thesis were, in my opinion, achieved.

I declare that defendant Ghazaleh Yassaghi proved to have the ability to perform research and to achieve scientific results and her thesis fulfils all requirements posed on theses aimed for obtaining Ph.D. degree. Therefore I do recommend the submitted thesis to be accepted for defense.

Questions:

Q1: Pages 87-90, Figure 3.3.6a and 3.3.7a: Why the $-\ln(\Sigma I_p/(I_p+\Sigma I_f))$ was used as Y-axis unit while in most other IRMPD spectra the non-logarithmic form $\Sigma I_p/(I_p+\Sigma I_f)$ was used?

Q2: What are the blue dotted lines in IRMPD spectra? I see that they correspond to certain wave numbers, but how their values were determined? For example those of C=C and C=O stretches in Figures 3.1.4a and 3.1.4c, and C=O stretches in Figures 3.1.6a and 3.1.6d.

Q3: Page 73, text referring to Figure 3.2.11: "The first band corresponds to the carbonyl stretch at 1609 cm^{-1} , ..." It is clearly seen in this Figure that band A in Figure 3.2.11a has in fact three maxima. Is there any explanation for that? And what are the two lines between 1600 and 1620 cm^{-1} in the calculated spectrum 3.2.11b? Does it mean that structure ${}^2\mathbf{3}_{\text{Me}}$ is not perfectly symmetric and those two lines correspond to two different carbonyls?

Q4: Though it is not explicitly indicated on which instrument the pressure dependent ion-molecule experiments in chapter 3.3.3 were performed, I assume it was on TSQ 7000. So my question is, was the baratron connected directly to the collision chamber? Can you show a picture describing some technical details?

Q5: Page 75-76, Figure 3.2.12: The elimination of CH_3COOH from ${}^2\mathbf{1}_{\text{Ace}}$ is rather complicated reaction in which three bonds are cleaved and two new bonds are formed. Is it a single-TS reaction? If yes, I would like to see the visualization of the corresponding ICR calculation or visualization of the vibration that corresponds to the imaginary frequency. If not, how do you know that the transition states shown in Figure 3.2.12 are the highest points on the respective reaction pathways?

Praha 23.1.2018

Mgr. Miroslav Polášek, Ph.D.