

Electrochemical generation of Cu(I) complexes in aqueous solutions studied by on-line mass spectrometry

Michel Prudent, Christophe Roussel, Hubert H. Girault *

Laboratoire d'Electrochimie Physique et Analytique, Ecole Polytechnique Fédérale de Lausanne, Station 6, 1015 Lausanne, Switzerland

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Abstract

Taking advantage of the electrochemical aspects of electrospray ionization (ESI) sources in positive ionization mode mass spectrometry (MS), aqueous copper complexes are electrogenerated using sacrificial copper electrodes. When using 8-hydroxyquinoline or bathocuproine, two chelating agents for Cu^{2+} and Cu^+ ions, respectively, the electrodisolution of copper leads to the formation of the respective complexes that are then analyzed by MS. This electrochemical generation of copper(I) complexes offers a direct route to synthesize Cu^+ complexes in aqueous solutions.

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1. Introduction

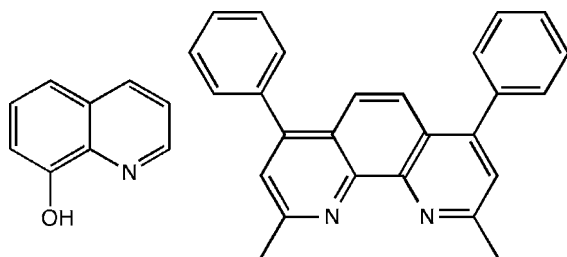
Transition metal ions and especially copper are essential elements in all living systems and act as electron transfer, dioxygen carrier or are involved in the oxidation of various substrate [1]. Mass spectrometry (MS), coupled to electrospray ionization (ESI), is a powerful tool to study biological and bioinorganic molecules [2]. By applying a high-voltage to a solution a fine aerosol of highly charged droplets is produced. This technique was first developed 80 years ago in different fields such as fuel atomization or electrostatic painting. Dole et al. used this technique to transfer high molecular weight polystyrene ions into gas-phase from solution [3], and in the 1980s Fenn and coworkers presented a series of papers that permanently established electrospray as a tool to introduce dissolved analytes into gas-phase for mass analysis [4].

In MS studies, Cu^+ has been produced by different ionization techniques [5–8] and by reduction of copper(II) salts during the ionization process [9,10]. Metallic copper

has been also used to generate Cu^+ ions in the gas-phase by direct ionization of the metal in matrix-assisted laser desorption ionization (MALDI) and fast atom bombardment (FAB) [11–13]. In ESI-MS, the electrode used to apply the high-voltage acts as an anode when operating in the positive ionization mode [14]. An electrospray can be viewed as an electrochemical flow cell, where the working electrode is in a solution and the counter electrode in the gas-phase. Taking advantage of the electrochemical aspects of ESI, copper ions can be electrogenerated in aqueous solutions. The use of sacrificial anodes in MS has been reported by Rohner and Girault [15] and van Berkel and Kertesz [16] mainly with the aim of tagging molecules with transition metal ions.

In the present work, we used electrospray microchips [17,18] where the electrode was made of copper, etching away during the electrospray. 8-hydroxyquinoline (Hox) and bathocuproine (BCP), specific ligands for Cu^{2+} and Cu^+ (Scheme 1), respectively, were electrosprayed with a sacrificial copper electrode. These experiments, confirmed by UV–vis experiments, highlight the electrogeneration of stable Cu^+ complexes in solution. The long term objective is to show that electrospray microchips with built-in copper

* Corresponding author. Tel.: +41 21 693 31 45; fax: +41 21 693 36 67.
E-mail address: hubert.girault@epfl.ch (H.H. Girault).



Scheme 1. 8-Hydroxyquinoline (Hox) and bathocuproine (BCP).

electrodes can be used to study biologically relevant copper(I) complexes.

2. Experimental

2.1. Chemicals

8-Hydroxyquinoline (Hox, $M = 145.16$ g/mol) was bought from Aldrich (Steinheim, Germany). 2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline (bathocuproine, BCP, $M = 360.45$ g/mol) was from Acros Organics (Geel, Belgium). Hydroquinone and cupric sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) were from Fluka (Büchs, Switzerland). Methanol was from Riedel-de-Haën (Seelze, Germany). Deionized water (18.2 M Ω cm) was prepared using a Milli-Q system from Millipore (Bedford, MA, USA). The ligands were used as received and diluted at a final concentration of 50 μM in 50/50 (v/v) MeOH/H $_2$ O. All the solutions were prepared daily.

2.2. MS setup and microspray interface

Metal ion on-line complexation was carried out using a microspray interface described by Rohner and Girault [15]. It consists of a single microchannel (45 $\mu\text{m} \times 120$ $\mu\text{m} \times 1$ cm) polyimide microchip developed by DiagnoSwiss SA (Monthey, Switzerland) [17,18]. A reservoir of polycarbonate ($\phi = 6.5$ mm, $h = 5$ mm) was fixed at the inlet of the microchannel. Sample was loaded ($V = 100$ μL) in the reservoir in which an electrode was immersed to apply the high-voltage with respect to the mass spectrometer. This electrode was made either of platinum wire for blank and copper salt experiments or of a copper plate ($S = 30$ mm 2). Both were polished and rinsed with methanol before each experiment.

An LCQ DUO ion trap mass spectrometer (Thermo, San Jose, CA, USA) was used in positive ion mode. The heated capillary was kept at 200 $^\circ\text{C}$. The commercial ESI interface was removed and the microchip was mounted on a plate fixed on the probe slide adapter of the mass spectrometer. After the MS power supply onset ($U = 3.5$ kV) the chip was moved closer to the entrance of the MS. The current, was set at 50 or 80 nA by adjusting the distance between the microspray outlet and the entrance of the MS, and monitored by a nanoamperometer. The ion optics parameters were kept constant for all the experi-

ments. The flow rate was estimated at 300 nL/min according to Morier et al. [19].

2.3. UV-vis spectroscopy

The formation of 2BCP-Cu^+ complexes in solution was recorded on an Ocean Optics UV-vis spectrometer (Chem 2000-UV-vis). BCP was diluted at 50 μM in 2 mL of 50/50 MeOH/H $_2$ O, in the presence or absence of 50 μM of CuSO_4 . The electrodisolution of copper was performed on an Autolab PGSTAT 12 (Ecochemie B.V.) in galvanostatic mode ($i = 10$ μA) in the solution of BCP at 50 μM . The pseudo-reference and counter electrodes were made of platinum wires and the working electrode was the metallic copper plate used in MS. After 30 min of electrolysis the concentration of copper ions will either be equal to 50 μM ($[\text{Cu}^{2+}]$) or 100 μM ($[\text{Cu}^+]$). If required, an excess of reducing agent (hydroquinone) [20] was used to reduce all the Cu^{2+} ions produced from the anodic dissolution of copper.

3. Results

3.1. 8-Hydroxyquinoline complexes

Fig. 1 shows the mass spectra of a solution of Hox (specific for Cu^{2+}) [21] electrosprayed in the presence of copper(II) salt with a platinum electrode (a) or directly with a copper electrode without any added salt (b). When using a copper(II) salt in a classical electrospray cell, one observes copper complexes with one and two ligands ($m/z = 224.9$ Th and $m/z = 352.1$ Th) and a polynuclear complex ($m/z = 558.0$ Th), as reported by Ross et al. [22]. These data result from the presence of Cu^{2+} ions in solution and corroborate the chelation of these copper(II) ions with oxygen and nitrogen [23]. The electrospray with the copper electrode shows an equivalent peak pattern. Electrogenenerated copper ions, produced from the anodic dissolution of copper, reacted in solution with the ligand. Hox- Cu^{2+} complexes were observed after a few minutes of spraying. Similar experiments with a copper electrode performed with 2,2'-bipyridyl and leucine [9] have also exhibited the formation of Cu^{2+} complexes.

3.2. Bathocuproine complexes

Bathocuproine is a well-known reagent for chelating cuprous ions [21]. It forms a yellow 2:1 complex with copper in a tetrahedral symmetry. BCP is typically used for the spectrophotometric determination of Cu^+ , for example formed by reduction of Cu^{2+} [24]. The ESI-MS analysis performed with a platinum electrode in the presence of CuSO_4 (50 μM) exhibited the formation of both 1:1 and 2:1 BCP:copper complexes (Fig. 2a). The main complex 2BCP-Cu^+ contains Cu^+ ion ($m/z = 783.2$ Th) even if only Cu^{2+} ions were originally present in solution. The 1:1 complex at $m/z = 440.7$ Th exhibited an unexpected m/z value and a zoom scan experiment was required. The mass spec-

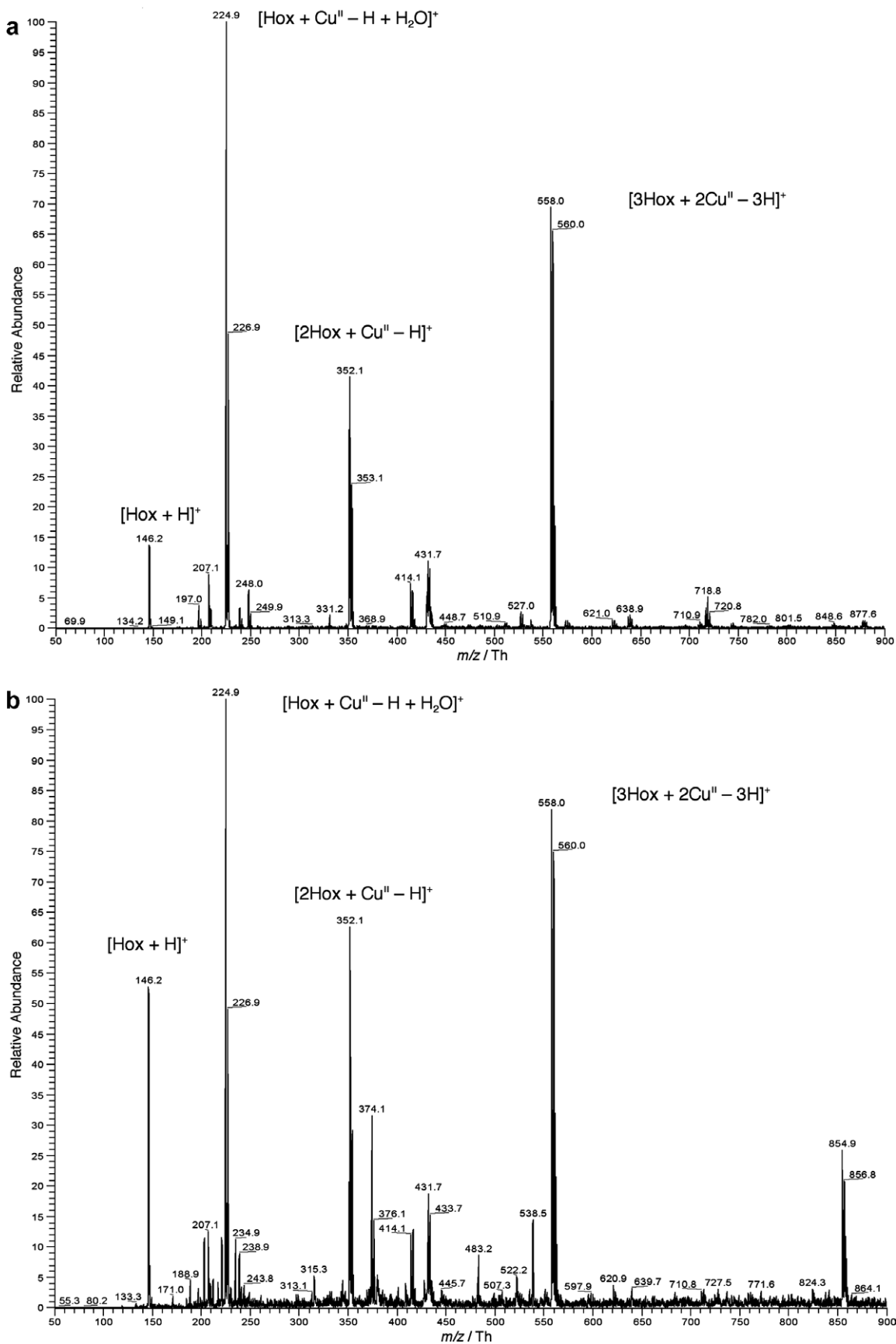


Fig. 1. Mass spectra of Hox (50 μ M) in 50/50 (v/v) MeOH/H₂O performed with (a) a Pt electrode in the presence of CuSO₄ at 50 μ M, $t = 7$ min and (b) a Cu electrode, $t = 8$ min. In both the experiments the complexes are mainly formed with Cu²⁺ ions.

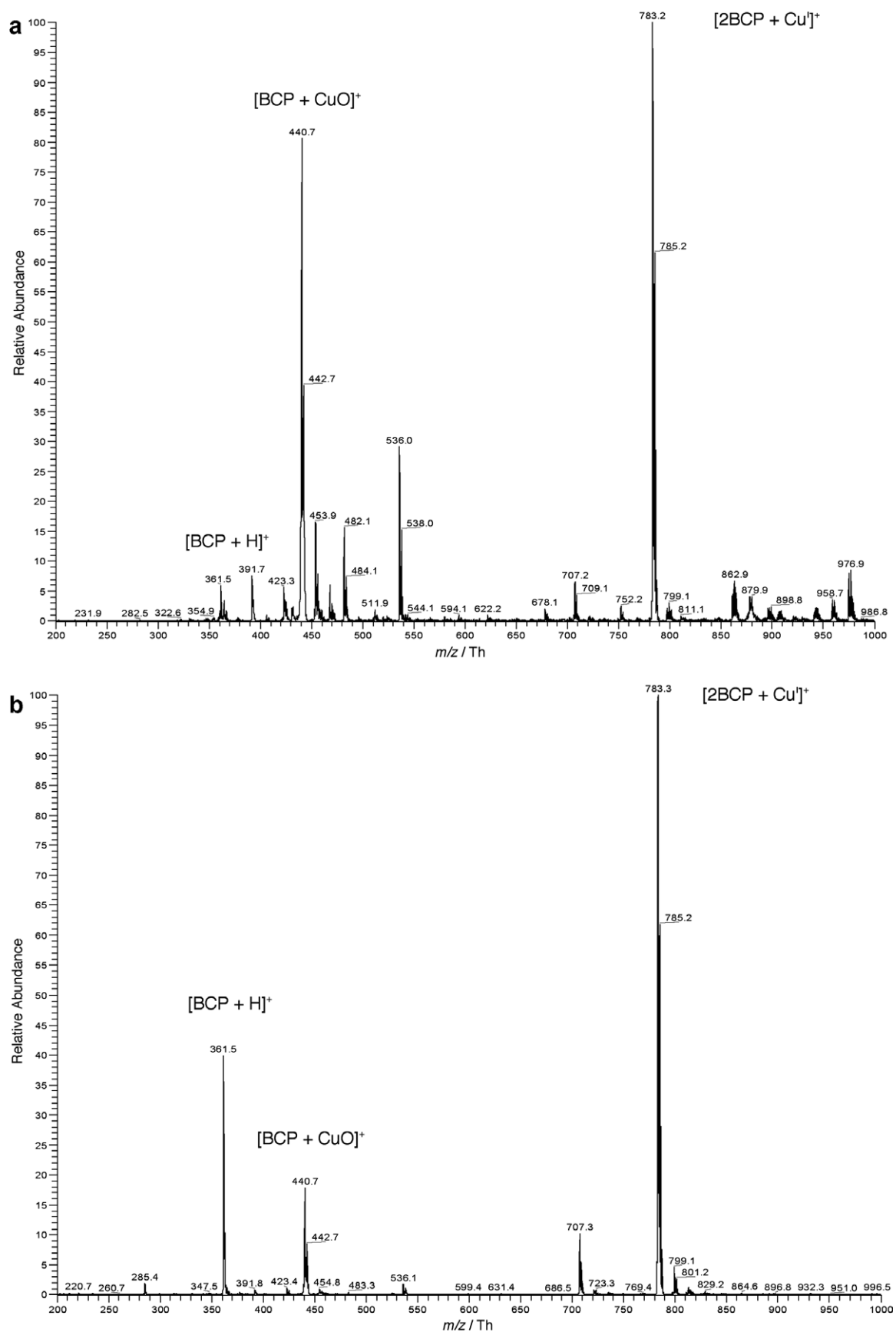


Fig. 2. Mass spectra of BCP (50 μ M) in 50/50 (v/v) MeOH/H₂O performed with (a) a Pt electrode in the presence of CuSO₄ at 50 μ M, $t = 11$ min and (b) a Cu electrode, $t = 12$ min. The mass spectra are different and the use of a copper electrode enhanced the 2BCP–Cu⁺ complex (see text for details).

trum shows mainly a peak at $m/z = 439.2$ Th. This mass-to-charge ratio corresponds to a ligated copper oxide cation: $[\text{BCP} + \text{CuO}]^+$. Schröder et al. have characterized this kind of complex with phenanthroline using copper(II) nitrate [25]. This compound stems from a Cu^{2+} complex in solution coordinated with nitrate and reduced in gas-phase. Here, Cu^{2+} is coordinated with H_2O or MeOH , which allows the formation of copper oxide cation. The experiments were repeated after adding an excess of reducing agent (hydroquinone) [20] to the solution prior to the electrospray to fully reduce Cu^{2+} and form mainly $2\text{BCP}-\text{Cu}^+$. The relative abundance of $[\text{BCP} + \text{CuO}]^+$ decreased from 81% to 14% and the formation of 2:1 complex was enhanced. These experiments clearly show that the complex $[\text{BCP} + \text{CuO}]^+$ is formed in the gas-phase by reduction of $\text{BCP}-(\text{H}_2\text{O})_n-(\text{MeOH})_m-\text{Cu}^{2+}$ complexes.

Fig. 2b shows the mass spectrum obtained after a few minutes of spray only with a copper electrode. The same complexes were observed as before, but the respective intensities were different. The major peak was the 2:1 complex whilst the 1:1 complex (corresponding to Cu^{2+} in solution) was less abundant (81% with CuSO_4 and 18% with copper electrode). As shown in Fig. 2a, only Cu^+ is observed in the mass spectrum. To show that copper(I) does not originate from a gas-phase reduction process and corroborate the Cu^+ formation in solution, UV-vis experiments were carried-out after electrodisolution of copper in the spray solution. Fig. 3 shows the absorbance of $2\text{BCP}-\text{Cu}^+$ complex (at 479 nm [21]) induced by anodic dissolution of copper (solid line). The $2\text{BCP}-\text{Cu}^+$ absorbance was higher than the Cu^+ complex formed following the dissolution of a copper(II) salt (dot line) in the spray medium. This small quantity of the 2:1 complex in solution after 30 min could come from reduction of Cu^{2+} complexes by methanol, where the reaction has a small kinetic constant, similar to those observed by Gianelli et al. [26].

As for the 1:1 complex, it has been shown that this cation stems from Cu^{2+} complex in solution and subsequently reduced. A small amount of Cu^{2+} seems to be produced during copper etching. To confirm this production during the anodic dissolution of copper in the presence of BCP, an excess of hydroquinone was added to the solution where Cu^+ ions were electrogenerated for UV-vis experiments. The absorbance (at 479 nm) increased to 0.17 a.u. (instead of 0.12 a.u.). These electrogenerated Cu^{2+} ions complexed by BCP and solvent molecules produced $\text{BCP}-\text{CuO}^+$ in gas-phase, albeit in lower quantity than the 2:1 complex.

3.3. Mixed-ligands complexation

Hox and BCP were then used together in the aim to compare the trend induced by ligands chelating competitively Cu^{2+} and Cu^+ ions. In the presence of a copper(II) salt (Fig. 4a), three complexes were observed: $\text{BCP}-\text{CuO}^+$ at $m/z = 440.7$ Th, $2\text{BCP}-\text{Cu}^+$ at $m/z = 783.2$ Th with a low relative abundance and a $\text{BCP}-\text{Hox}-\text{Cu}^{2+}$ complex

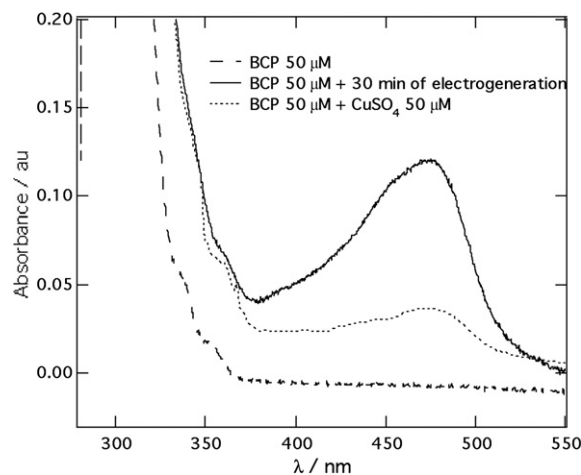
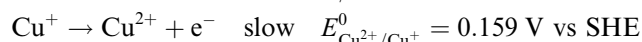
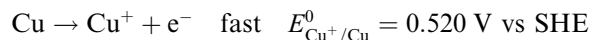


Fig. 3. Absorption spectra of $2\text{BCP}-\text{Cu}^+$ complex in 50/50 (v/v) MeOH/ H_2O . $[\text{BCP}] = 50 \mu\text{M}$. Spectrophotometric response of copper complex after the anodic dissolution of the copper electrode, and in the presence of CuSO_4 at $50 \mu\text{M}$.

at $m/z = 567.1$ Th. The experiment carried-out with the copper electrode (Fig. 4b) showed the formation of same complexes but again with different relative abundances. The $2\text{BCP}-\text{Cu}^+$ complex was the most important corroborating that specific ligands can react with electrogenerated Cu^+ in aqueous solutions. Moreover, a new $\text{BCP}-\text{Hox}-\text{Cu}^+$ complex appeared at $m/z = 568.3$ Th. Indeed, focusing on the isotopic distribution near this mass-to-charge ratio (see inserts), an overlap of two isotopic distributions was observed. The one at $m/z = 567.1$ Th corresponds to Cu^{2+} complex whereas the other at $m/z = 568.3$ Th corresponds to Cu^+ complex (not observed with the copper salt). In addition to the change in peak pattern, the electrochemical approach offers access to different complexes in solution, either Cu^{2+} or Cu^+ , which differed from other reported methods.

4. Discussion

The most commonly accepted mechanism for copper etching is that proposed in 1959 by Mattsson and Bockris [27–29]:



The detachment of Cu^+ ions from the metal is considered as a fast process, whereas the second oxidation step is slower involving the oxidation of a hydrated species. Cu^+ is thermodynamically unstable in aqueous solution in the absence of ligands such as chloride or nitrogen- or sulfur-based ligands [23] and is further oxidized at the electrode. In the presence of specific ligands, the complex formation will decrease the oxidation potential of Cu^+/Cu whilst increasing that of $\text{L}-\text{Cu}^{2+}/\text{L}-\text{Cu}^+$, and thereby copper etching will preferentially produce Cu^+ complexes (Scheme 2).

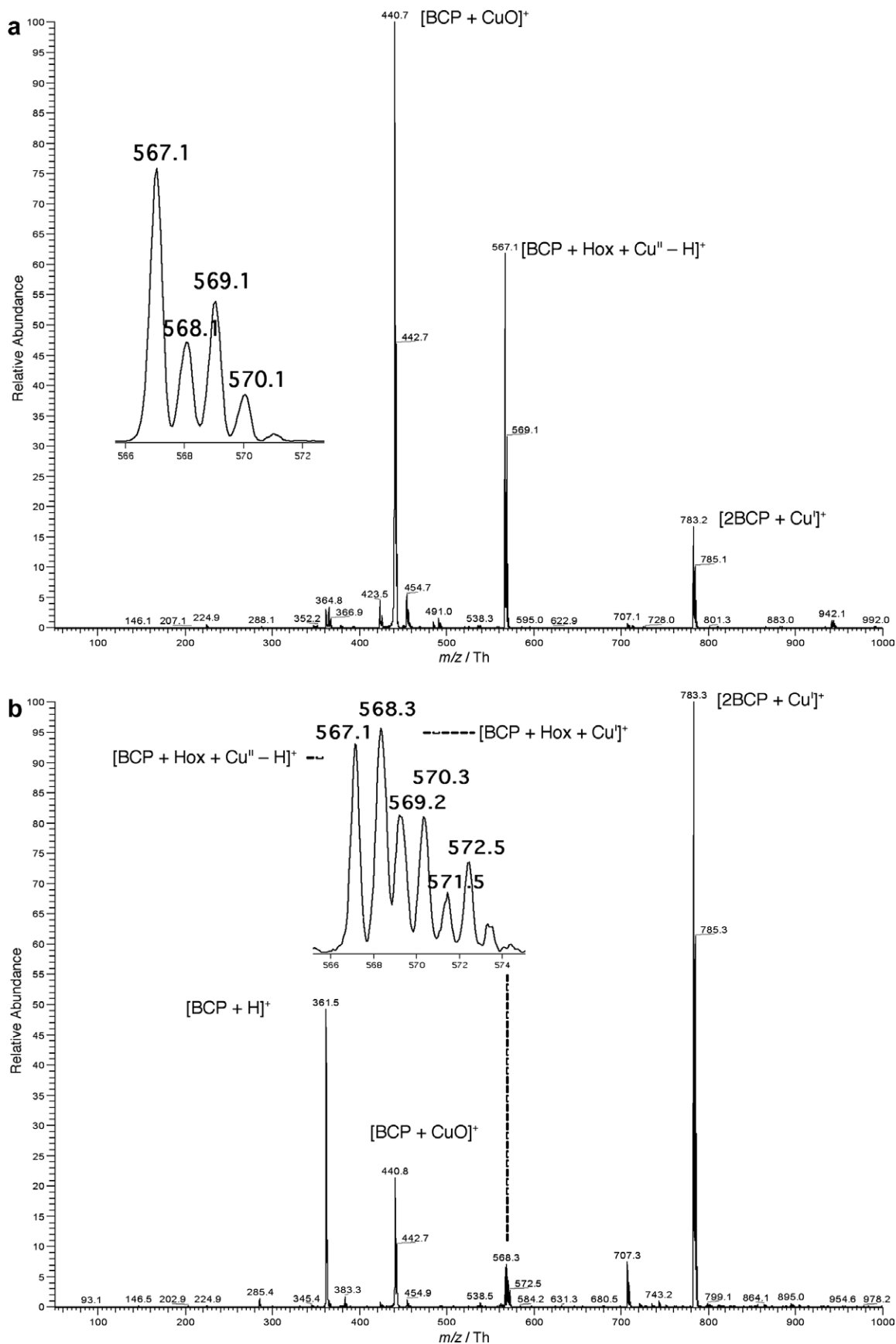
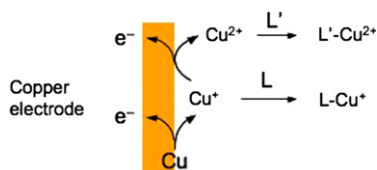


Fig. 4. Mass spectra of a mixture of Hox (50 μM) and BCP (50 μM) in 50/50 (v/v) MeOH/H₂O performed with (a) a Pt electrode in the presence of CuSO₄ at 50 μM , $t = 6$ min and (b) a Cu electrode, $t = 7$ min. Depending on the behavior of the ligands, the experiments give different peak patterns. The insets show the isotopic distribution of the BCP-Hox-Cu^{II} complexes. A Cu⁺ complex is formed in (b), thanks to the use of a Cu electrode.



Scheme 2. Copper oxidation pathways according to ligands present in solution.

As shown by Hox experiments, Cu^{2+} was produced by anodic dissolution of copper and reacted on-line with the ligand. The results were equivalent to those obtained with copper(II) salt. However, according to Faraday's law, the concentration of Cu^{2+} was $2\ \mu\text{M}$ after 8 min of electro-spray. This concentration was lower than the concentration of Hox ($50\ \mu\text{M}$) and CuSO_4 ($50\ \mu\text{M}$) in Fig. 1a. However, the ratio between the complexes and the native peak ($[\text{Hox}+\text{H}]^+$) appears higher in comparison with the ratio of concentrations. This phenomenon cannot only be explained by the ionization efficiency, but more by the absence of counter ions (such as sulfate) when using a sacrificial electrode.

By using a specific ligand, Cu^+ complexes were observed. The formation of these complexes comes from either the reduction of Cu^{2+} or the oxidation of copper electrode. When a copper(II) salt is present in solution, Cu^{2+} can be reduced in the gas-phase. Lavanant et al. have observed the reduction of Cu^{2+} complexes by electron capture [10]. Cu^{2+} reduction can be favored by increasing the nozzle-skimmer voltages or the source voltage. Schröder et al. have also observed this phenomenon by increasing the cone voltage tension [25]. Gianelli et al. have reported the effect of the presence of solvents on Cu^{2+} reduction and correlated the production of Cu^+ with the ionization energy of alcohols [26]. Cu^+ ions formed in the gas-phase can react with ligands offering a better affinity for Cu^+ . As a conclusion, in the presence of copper(II) salt both 2:1 complex and 1:1 complex come from the reduction of Cu^{2+} complexes.

When spraying BCP with a sacrificial copper electrode, two mechanisms can explain the formation of Cu^+ complexes. Gas-phase reactions reducing Cu^{2+} to Cu^+ as described above, or the anodic dissolution of copper electrogenerating Cu^+ ions, which are stabilized by specific ligands. Referring to the UV-vis experiments, the $2\text{BCP}-\text{Cu}^+$ was produced in solution. Moreover, these data indicate the formation of a small quantity of Cu^{2+} ions. The anodic dissolution of copper involves a two-step reaction. Each uncomplexed Cu^+ ions are further oxidized at the copper electrode. Then, Cu^{2+} ions can react with ligands and solvent molecules. We can therefore conclude unambiguously that Cu^+ ions are produced within the ESI interface by copper electrooxidation and not only by gas-phase reactions. The mass spectrum in Fig. 4b highlights the formation of $\text{BCP}-\text{Hox}-\text{Cu}^+$ whereas no reduction phenomena could produce this complex with a copper(II) salt.

It is important to stress that the electrospray reactions are carried out in the absence of added salt. The copper dissolution occurs in the absence of counter anions, such as sulfate or chloride, known to have some effects on copper etching and in positive ionization MS [30].

5. Conclusions

The present mass spectrometry study clearly shows that Cu^+ ions can be produced in aqueous solutions during the electrochemical oxidation of copper metal. Without adding any salts the experiments carried-out with a copper electrode gave equivalent results (with lower Cu^{2+} concentration) to those carried-out with copper salts, as proved by 8-hydroxyquinoline-copper complexes. Moreover, this technique offers the access to Cu^+ ions in solution, without specific preparation of the sample, thanks to the mechanism of the anodic dissolution of copper, as shown by bathocuproine experiments and yielding the production of a $\text{BCP}-\text{Hox}-\text{Cu}^+$ complex. Even if redox phenomena occur in gas-phase, UV-vis experiments confirmed the electrogeneration of stable Cu^+ complexes in solution.

More generally, these results show that electrospray ionization carried out with a copper electrode offers a new route to study aqueous Cu^+ complexes, for example to study Cu^+ -peptide complexes. The study of these interactions with sacrificial copper electrodes is under investigations.

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