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Research Article

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Unexpected formation of $[M]^{2+}$ from $[M+CuCl+H]^{2+}$ ions under CID conditions, where M is a molecule of 3,5-bis(2,2'-bipyridin-4-ylethynyl)benzoic acid or its methyl ester

Abstract: $[M+CuCl+H]^{2+}$ ions were generated using electrospray ionization (ESI); where M is a molecule of 3,5-bis(2,2'-bipyridin-4-ylethynyl)benzoic acid or its methyl ester (1 and 2, respectively). The ions were subjected to CID-MS/MS analysis. It was found that their gas phase decomposition lead to the formation of rare di-cations $[M]^{2+}$, namely $[1]^{2+}$ and $[2]^{2+}$ ions. The formation of $[1]^{2+}$ ion from $[3+H+CuCl]^{2+}$ ion in the second fragmentation, where 3 is ethyl ester of 3,5-bis(2,2-bipyridin-4-ylethynyl)benzoic acid, was also observed since in the first fragmentation step the loss of ethylene molecule from $[3+H+CuCl]^{2+}$ ion took place. To the best of our knowledge, it is the first time that $[M]^{2+}$ ions formation from respective metal complexes has been reported. It is also unusual that formation of $[M]^{2+}$ ions is not accompanied by formation of $[M]^{+\bullet}$ ions. Furthermore, as expected, theoretical calculation and electron ionization mass spectra show that 1 and 2 are not especially prone to form $[M]^{2+}$ ions. Thus formation of $[M]^{2+}$ ions under CID conditions is very surprising.

Keywords: di-cation, copper complexes, electrospray ionization mass spectrometry, collision induced dissociation

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1 Introduction

Under electron ionization (EI) conditions, singly charged organic cations $[\mathbf{M}]^{+\bullet}$, formally radical cations, are formed (**M** stands for a molecule of organic compound). These ions, also called molecular ions, are formed by the loss of one electron from the neutral organic molecule. Observations of the loss of two electrons leading to doubly charged ions $[\mathbf{M}]^{2+}$ (di-cations) under EI conditions have been very rare [1-6]. Under electrospray ionization (ESI) conditions, organic radical cations $[\mathbf{M}]^{+\bullet}$ also can be formed, and this phenomenon is quite well known. Under ESI conditions, $[\mathbf{M}]^{+\bullet}$ ions can be formed as a results of gas phase decomposition of respective metal-**M** complexes, mainly Cu-**M** complexes [7-13]. Ions $[\mathbf{M}]^{+\bullet}$ can be also formed (under ESI conditions) from compounds with low oxidation potential through their oxidation by capillary voltage [14-16]. There are also rare examples of formation of ions $[\mathbf{M}]^{2+}$ as a result of ESI oxidation process [17-21].

However, to the best of our knowledge, there are no examples of $[\mathbf{M}]^{2+}$ ions formation from respective metal-**M** complexes. In this paper, we report the formation of $[\mathbf{M}]^{2+}$ ions from $[\mathbf{M}+H+CuCl]^{2+}$ ions, upon gas phase decompositions of the latter, where **M** is 3,5-bis(2,2-bipyridin-4-ylethynyl)benzoic acid or its methyl ester, results obtained for ethyl ester are also included (**1**, **2** and **3**, Fig. 1).

2 Experimental

Compounds **1**, **2** and **3** (Fig. 1) were prepared as described elsewhere [22].

ESI-CID-MS/MS spectra were taken on a Waters/Micromass (Manchester, UK) Q-tof Premier mass spectrometer (software MassLynx V4.1, Manchester, UK).

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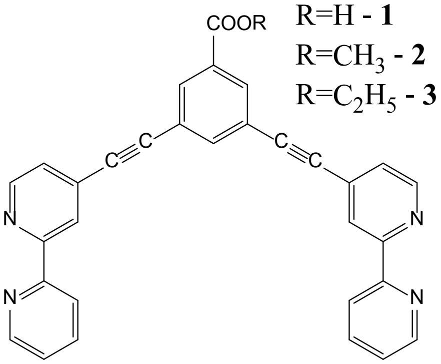


Figure 1: Structure of 3,5-bis(2,2-bipyridin-4-ylethynyl)benzoic acid - compound **1** and its methyl and ethyl esters (**2** and **3**).

The sample solutions were infused into the ESI source by a syringe pump at a flow rate of 5 µl min^{-1}. The electrospray voltage was 2.7 kV and the cone voltage – 30 or 50 V. Cone voltage has the most profound effect on the full scan mass spectra obtained. An increase in this parameter leads to the so called, "in-source" fragmentation/dissociation, but a too low cone voltage could cause a decrease in sensitivity. At higher cone voltage of 50 V, [**M**+H+Cu]$^{2+}$ ions were generated and then their CID-MS/MS spectra were obtained. The source temperature was 80°C and the desolvation temperature was 250°C. Nitrogen was used as the cone gas and desolvating gas at the flow-rates of 50 and 800 l h^{-1}, respectively. Argon was used as a collision gas at the flow-rate of 0.5 ml min^{-1} in the collision cell. The applied collision energy (CE, laboratory frame), the most important parameter for CID-MS/MS experiments, is indicated in each CID-MS/MS spectrum shown.

Accurate mass measurements of [**1**]$^{2+}$ and [**2**]$^{2+}$ ions were made using the same instrument. Known accurate masses of the precursor ions ([**1**+H+CuCl]$^{2+}$ and [**2**+H+CuCl]$^{2+}$ ions) were used as lock masses. The experimentally obtained values were 239.0734 for [**1**]$^{2+}$ and 246.0815 for [**2**]$^{2+}$. The exact calculated values are 239.0715 and 246.0793, respectively. Thus, the obtained values have confirmed the elemental compositions $C_{31}H_{18}O_2N_4$ and $C_{32}H_{20}O_2N_4$, respectively.

Electron ionization mass spectra (70 eV) were recorded on a Bruker 320-MS triple quadrupole mass spectrometer; the compounds were introduced by using a direct inlet probe.

Calculation of ionization potentials are as follows: Structures of the studied compounds neutral, bearing +1 and +2 charge were optimized with the latest functional from Head-Gordon and coworkers, which included empirical dispersion (wb97xd) [23] and 6-31++G(d,p) basis set, as wb97xd functional was indicated in recent studies "to be just right for conjugated systems at all chain lengths" [24]. In the excited states, doublet state of +1 cations, and both singlet and triplet states of +2 cations were considered. Ionization potential – as defined – is the amount of energy required to remove an electron from molecule. The suitable adiabatic ionization potentials were calculated as the difference between the energies of the appropriate cationic forms and neutral ones at their optimized geometries [25]. The vertical ionization potentials were calculated as the difference between the energies of the appropriate cation forms and neutral ones at optimized neutral geometries [25]. The calculations of all the structures were performed with Gaussian09 [26].

3 Results and discussion

Recently we have published the results of our ESI-MS study on copper complexes of 3,5-bis(2,2-bipyridin-4-ylethynyl)benzoic acid – (**1**) and its methyl and ethyl ester (**2** and **3**) [27] (Fig. 1). For the solutions containing the same concentrations of ligand (**M** = **1**, **2**, **3**) and $CuCl_2$, the most abundant ions corresponded to the protonated ligand molecules, [**M**+H]$^+$ ions; copper complexes were also detected but less abundant (e.g. [$\mathbf{M}_2$+CuCl]$^+$). For the solution containing three-fold excess of $CuCl_2$, [**M**+H]$^+$ ions were not detected, and the most abundant cations were doubly charged ions [**M**+Cu_2Cl_2]$^{2+}$ [27].

As expected, by adjusting **M** and $CuCl_2$ concentrations, it is possible to generate ions composed of **M**, proton and copper, namely [**M**+H+CuCl]$^{2+}$ ions. Formation of these ions is not especially interesting. On the other hand, we found that upon gas phase decompositions of these ions, unexpectedly, doubly charged [**M**]$^{2+}$ ions are formed. Fig. 2 shows ESI-CID-MS/MS spectra of [**1**+H+CuCl]$^{2+}$ and [**2**+H+CuCl]$^{2+}$ ions, ions [**1**]$^{2+}$ and [**2**]$^{2+}$ are not very abundant but clearly seen. The obtained isotope patterns and exact masses confirmed the presence of [**1**]$^{2+}$ and [**2**]$^{2+}$ ions.

The most unusual is that there are no [**M**]$^{+\bullet}$ ions but there are [**M**]$^{2+}$ ions. Taking into account the calculated ionization potentials of **1** and **2**, the compounds are not especially prone to form [**1**]$^{2+}$ and [**2**]$^{2+}$ ions and do not form [**1**]$^{+\bullet}$ and [**2**]$^{+\bullet}$ ions (Table 1).

The question is how [**1**]$^{2+}$ and [**2**]$^{2+}$ ions are formed from [**M**+H+CuCl]$^{2+}$ ions, in other words, what is the pathway of [**M**]$^{2+}$ ions formation? It may be that [**M**]$^{2+}$ fragment ions are formed directly from parent ions [**M**+H+CuCl]$^{2+}$ ions or are formed in the second fragmentation step, from other fragment ions.

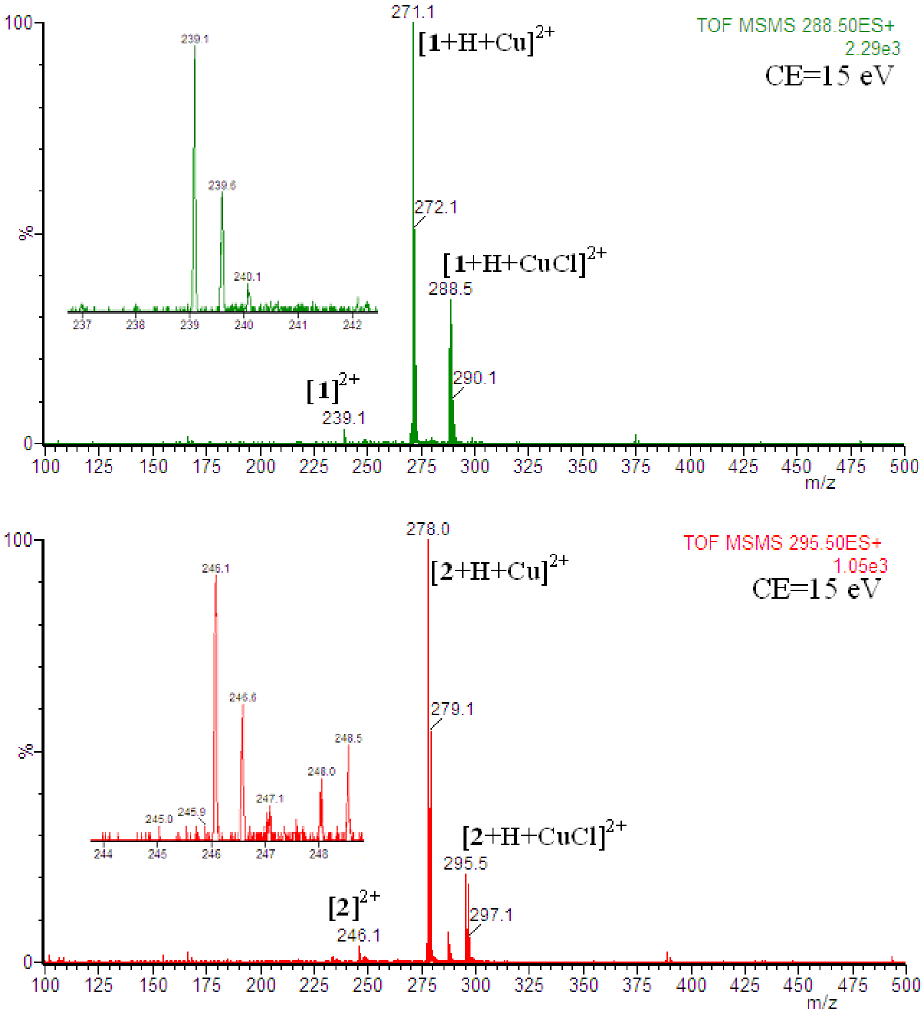


Figure 2: ESI-CID-MS/MS spectra of [**1**+H+CuCl]$^{2+}$ and [**2**+H+CuCl]$^{2+}$ ions.

Table 1: Adiabatic and vertical ionization potentials (IP, eV) of **1**, **2** and **3**.

Compound	adiabatic first IP	adiabatic second IP	vertical first IP	vertical second IP
1	8.07	17.94 (triplet state) 19.65 (singlet state)	8.23	18.31 (triplet state) 19.93 (singlet state)
2	7.97	18.04 (triplet state) 19.52 (singlet state)	8.22	18.25 (triplet state) 19.91 (singlet state)
3	7.97	18.04 (triplet state) 19.49 (singlet state)	8.13	18.24 (triplet state) 19.79 (singlet state)

The main decomposition of [**M**+H+CuCl]$^{2+}$ ions consist of the loss of Cl atom, producing [**M**+H+Cu]$^{2+}$ ions (formally red-ox process, Cu is reduced, Cl is oxidized). Further decomposition of [**M**+H+Cu]$^{2+}$ ions (loss of Cu and H atoms, or CuH molecule) may lead to the formation of [**M**]$^{2+}$ ions. However, we found that [**M**]$^{2+}$ ions are not formed from [**M**+H+Cu]$^{2+}$ ions. It was possible to generate [**M**+H+Cu]$^{2+}$ ions by the so-called dissociation “in-source” and then obtain their CID-MS/MS spectra. Fig. 3 shows a CID-MS/MS spectrum of [**1**+H+Cu]$^{2+}$ ion as an example.

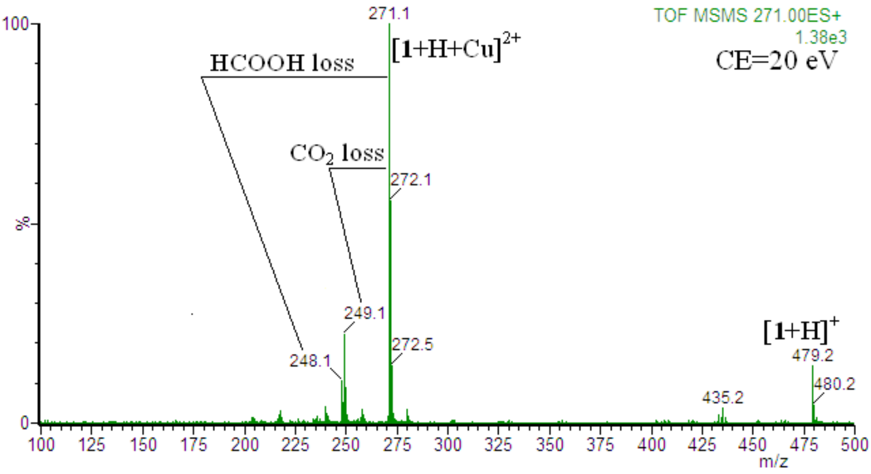


Figure 3: CID-MS/MS spectrum of [**1**+H+Cu]$^{2+}$ ion.

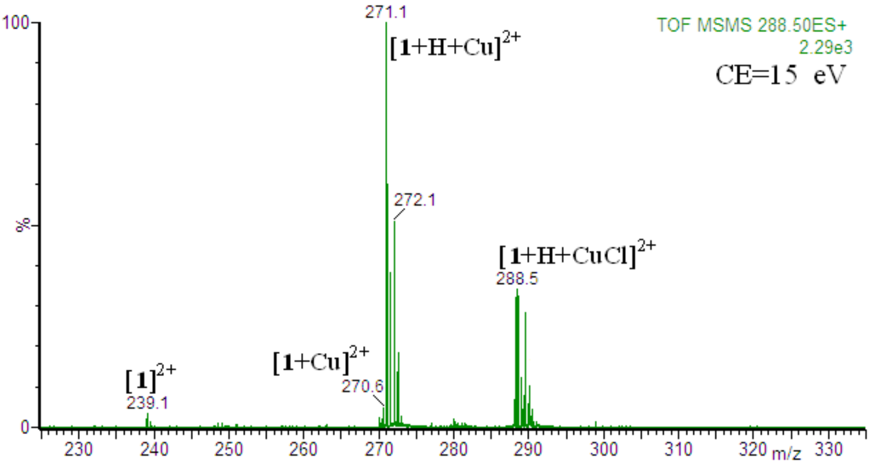


Figure 4: CID-MS/MS spectrum of [**1**+H+CuCl]$^{2+}$ ion in the m/z range of interest. [**1**+Cu]$^{2+}$ ion formed by the loss of HCl molecule is seen.

Decomposition of [**1**+H+Cu]$^{2+}$ ion consists of the loss of CO_2 and HCOOH molecules and formation of [**1**+H]$^{+}$ ion, but formation of [**1**]$^{2+}$ is not observed.

After careful examination of CID-MS/MS spectra of [**M**+H+CuCl]$^{2+}$, we found that in addition to the loss of Cl atoms, the loss of HCl molecules as well, produces [**M**+Cu]$^{2+}$ ions, and takes place as shown in Fig. 4.

We have tried to obtain of CID-MS/MS spectra of [**M**+Cu]$^{2+}$ ions; however, these ions had very low abundances on the full scan mass spectra. The selected ion beam always contained a lot of [**M**+H+Cu]$^{2+}$ ions and the obtained spectra looked identical as those of [**M**+H+Cu]$^{2+}$ ions with [**M**]$^{2+}$ ions not detected. Furthermore, formation of [**M**]$^{2+}$ ions from [**M**+Cu]$^{2+}$ ions cannot be expected without formation of [**M**]$^{+\bullet}$ ions [6,28].

Thus, it reasonable to assume that [**M**]$^{2+}$ ions are formed directly from [**M**+H+CuCl]$^{2+}$ ions. It is difficult to propose what neutral species are lost from [**M**+H+CuCl]$^{2+}$ ions (there are few possibilities), since respective intermediates were not observed.

It has to be stressed that a number of ions were subjected to the CID-MS/MS analysis (among others [**M**+Cu_2Cl]$^{2+}$ ion, this ion can be regarded as the most similar to the [**M**+H+CuCl]$^{2+}$ ion); however, [**M**]$^{2+}$ ions were formed only from [**M**+H+CuCl]$^{2+}$ ions. It is difficult to rationalize why [**M**]$^{2+}$ ions are formed from [**M**+H+CuCl]$^{2+}$ ions in the gas phase, under CID conditions.

Results obtained for **3** (ethyl ester of **1**, Fig. 1) were different from the above discussed results obtained for **1** and **2**. Namely, the decomposition of [**3**+H+CuCl]$^{2+}$ ion did not yield [**3**]$^{2+}$ ion, but led to the formation of fragment ion at m/z 239 which can be assigned as [**1**]$^{2+}$ ion, as shown in Fig. 5.

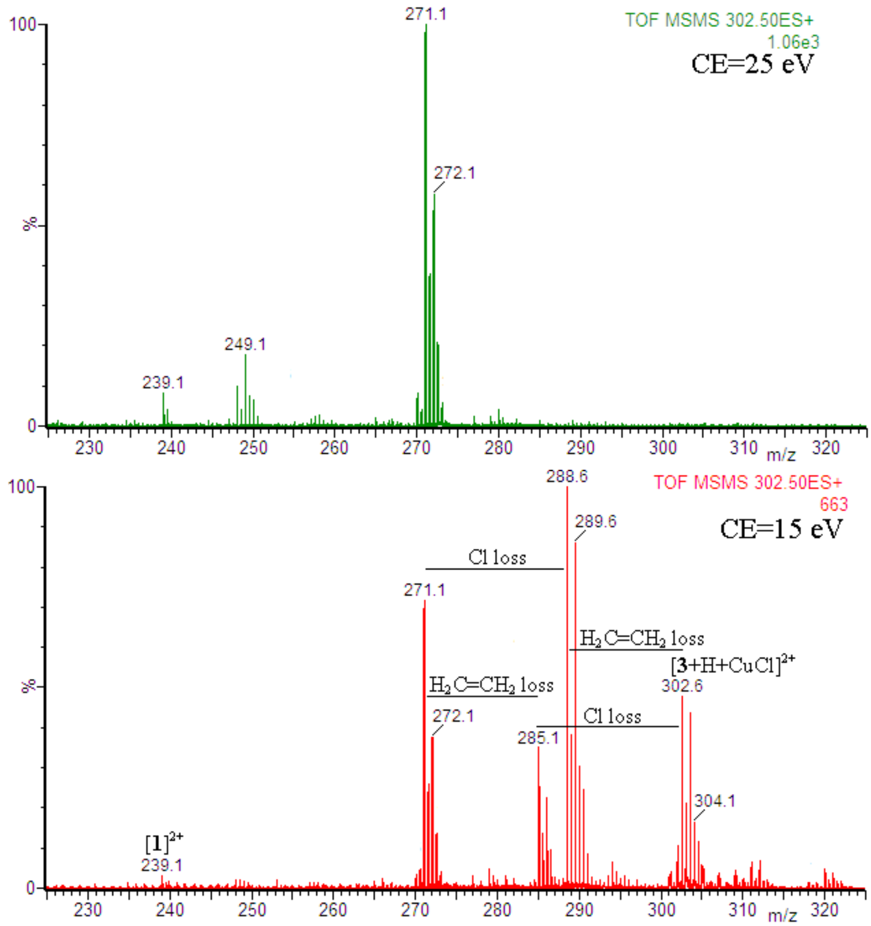
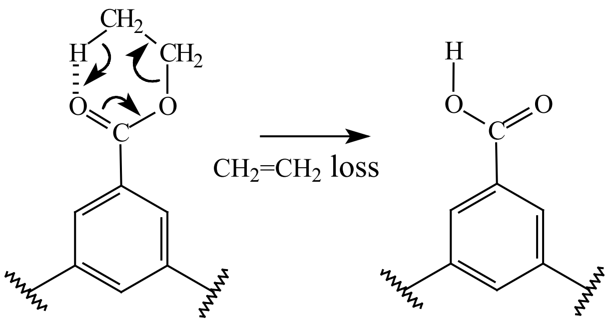


Figure 5: CID-MS/MS spectra of [**3**+H+CuCl]$^{2+}$ ion shown in the m/z range of interest. At collision energy of 15 eV, there is abundant fragment ion formed by the loss of an ethylene molecule from [**3**+H+CuCl]$^{2+}$ ion. At collision energy of 25 eV, the fragment ion at m/z 239, which may be assigned as [**1**]$^{2+}$, is quite abundant.



Scheme 1: Loss of an ethylene molecule through McLafferty's rearrangement.

In the first fragmentation step, [**3**+H+CuCl]$^{2+}$ ion loses an ethylene molecule producing fragment ion at m/z 288.5 (ion can be assigned as [**1**+H+CuCl]$^{2+}$). In the second fragmentation step, the [**1**]$^{2+}$ ion is formed from the ion at m/z 288.5. The loss of the ethylene molecule occurs through the six-membered ring, as shown in Scheme 1, and can be regarded as the so-called McLafferty rearrangement [29].

It is interesting to check if, under EI conditions, the [**M**]$^{2+}$ ions can be formed from compounds **1**-**3**. EI conditions are regarded as the so-called "hard" conditions, since electron beam has the energy of 70 eV (standard value). Fig. 6 shows EI mass spectra of **1**-**3**.

As shown in Fig. 6, the [**M**]$^{2+}$ ions were detected but were characterized by low abundances (m/z 239, 246, 253). The spectrum of **3** shows a low abundant [**3**]$^{+}$ ion (m/z 253). Thus, compounds **1**-**3** are not especially prone to form [**M**]$^{2+}$ ions, as it also results from the calculated ionization potentials (Table 1). Thus, formation of [**M**]$^{2+}$ ions from [**M**+H+CuCl]$^{2+}$ ions under CID conditions is even more interesting if, not even, strange.

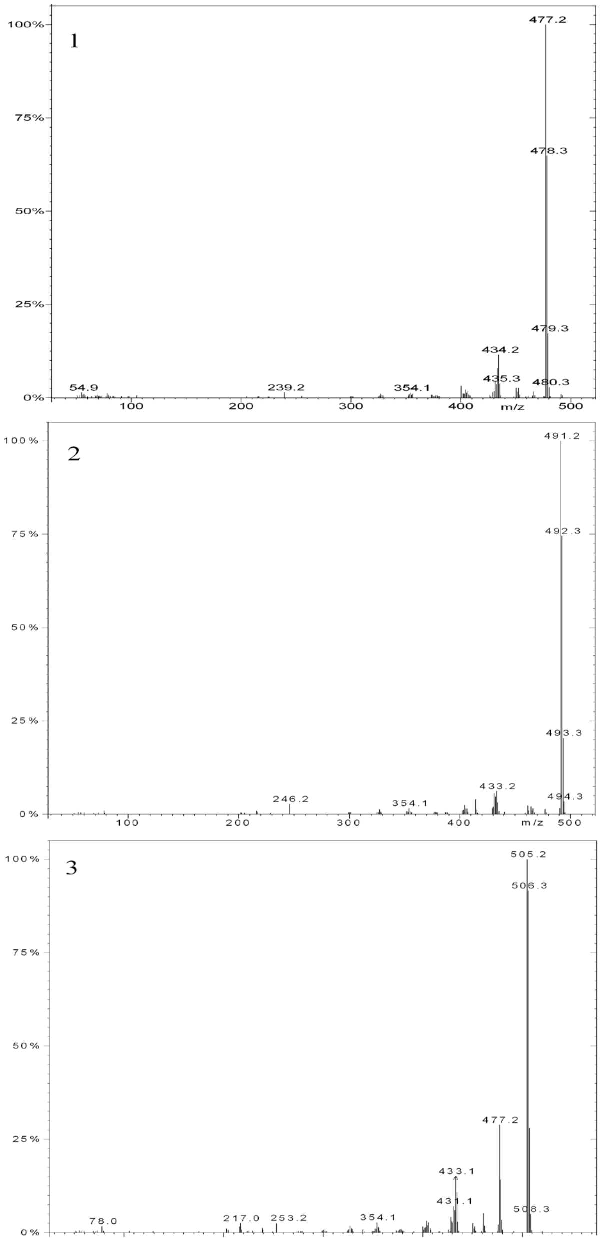


Figure 6: EI mass spectra of **1-3**.

4 Conclusion

Formation of $[\mathbf{1}]^{2+}$ and $[\mathbf{2}]^{2+}$ ions was observed as a result of gas phase decomposition of $[\mathbf{1}+H+CuCl]^{2+}$ and $[\mathbf{2}+H+CuCl]^{2+}$ ions, where **1** and **2** are 3,5-bis(2,2-bipyridin-4-ylethynyl) benzoic acid and its methyl ester, respectively. To the best of our knowledge, it is the first time that $[\mathbf{M}]^{2+}$ ions have been observed to form from respective metal complexes. The observed formation of $[\mathbf{M}]^{2+}$ ions has to be regarded as a feature of decomposition of $[\mathbf{M}+H+CuCl]^{2+}$ ions since $[\mathbf{M}]^{2+}$ ions were formed only from them. It is also unusual that formation of $[\mathbf{M}]^{2+}$ ions is not accompanied by formation of $[\mathbf{M}]^{+\bullet}$ ions. The formation of $[\mathbf{1}]^{2+}$ ion from $[\mathbf{3}+H+CuCl]^{2+}$ ion in the second fragmentation step, where **3** is ethyl ester of 3,5-bis(2,2-bipyridin-4-ylethynyl)benzoic acid, was also observed since in the first fragmentation step, the loss of ethylene molecule from $[\mathbf{3}+H+CuCl]^{2+}$ ion took place.

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