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Electrospray Mass Spectrometry of Electrochemically Ionized Molecules : Application to the Study of Fullerenes.

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Abstract: We describe the characterization, by Electrospray Ionization Mass Spectrometry (ESI-MS), of neutral species using their redox properties. These molecules were ionized by oxidation-reduction reactions either off-line, in a classical electrochemical cell, or on-line using the fact that the ES source behaves like an electrolysis cell. This study has been performed both for oxidation (positive ions) and reduction (negative ions) reactions. Limitations as to the ability of compounds to be analyzed on-line have been examined as have limitations of off-line reduction in the case of fullerenes. This study offers a new approach for the ESI-MS analysis of compounds difficult to ionize.

Since its discovery in the early 80's, Electrospray Ionization Mass Spectrometry (ESI-MS) has been intensively used for the characterization of peptides and proteins of high molecular weight (up to 100 kDa),[1] owing to its ability to induce the formation of multiply-charged ions, by protonation or deprotonation processes.[2]

The use of ESI-MS is now being extended to the characterization of non peptidic molecules, provided that a charge can be conferred on them. Much effort has been made especially in the field of coordination and supramolecular chemistry,[3a-b] where structural characterization is often difficult. In these fields, ionization is mainly due to the loss of one or several counterions present in the molecule.[3a-b] In some very special cases, the encapsulation of a charged guest molecule can allow the ionization of molecules such as methylated β-cyclodextrins.[4]

Despite this variety of ionization processes, many types of compounds still cannot be analyzed by ESI-MS. Therefore, it is worth developing ionization strategies for molecules that cannot be ionized according to the mechanisms described above.

Taking advantage of the redox properties of some molecules by donating one electron in a reduction process (negative ions) or by removing one electron via an oxidation process (positive ions) would provide access to ionization for ESI-MS. Only a few publications[5a-c,6] mention such studies. We believe that this approach will make possible the ESI-MS analysis of large coordination compounds with a high number of counterions, which decompose when the counterions are removed because of excessive coulombic repulsion.[7] Indeed, the addition of electrons would decrease these coulombic effects by reducing the number of charges on the species. Such a study is under way in our laboratory.

In this paper, we have first investigated a series of neutral compounds by ESI-MS without any previous electrolysis, in order to check whether the ES source behaves like an electrolysis cell as previously described.[6] We investigated oxidizable compounds with different redox potentials, as Xu *et al.*[8] did for metallocenes, and then extended this study to reducible compounds which, to our knowledge, is without precedent. In both cases, we tested whether the ability of a compound to undergo redox processes in the ES source can be predicted from the value of its redox potential. It is obvious that compounds with redox potentials beyond the limits established in this paper can be pre-ionized by off-line electrolysis and analyzed by ESI-MS, provided that the corresponding reduced or oxidized species is stable enough. In a next step we compared off-line electrochemical treatment with spontaneous oxido-reduction processes, in order to be able to evaluate problems which may arise when using one or the other method. The study has been performed on fullerene in the negative mode (reduction reactions). This work provides a new possibility for characterizing this typical, neutral compound by mass spectrometry. Indeed, although the characterization of fullerenes by mass spectrometry has been widely investigated by several ionization techniques such as electron ionization,[9] laser desorption,[10] FAB[10], the use of ESI-MS would be useful, especially for the characterization of higher homologues. Indeed, the other ionization techniques may induce either degradation or aggregation of the fullerenes, preventing the characterization of higher homologues really present in the sample.

EXPERIMENTAL :

Electrospray Ionization Mass Spectrometry:

ESI-MS was carried out on a BioQ (VG Bio Tech, Altrincham, UK). Experimental conditions were similar to those described earlier.[3b] Resolution was about 800 and the accelerating cone voltage Vc[3b] was set at 50V.

Sample preparation.

- The fullerene anions were generated by electrochemical reduction of fullerite (Aldrich, about 90% C_{60}, 10% C_{70}), dissolved at a concentration of 10^{-4} M in anhydrous CH_2Cl_2 with 10^{-2} M tetra(*n*-butyl)ammonium hexafluorophosphate (TBA-PF_6) as supporting electrolyte. Intensiostatic reduction was performed at 100 µA, and potentiostatic reduction at -0.70 V (first stage) or -1.20 V (second stage).
- The other compounds studied (Aldrich, Fluka or Sigma) were dissolved at a concentration of 10^{-5} M, 10^{-4} M, and 10^{-3} M in anhydrous CH_2Cl_2. Solutions were carefully degassed under argon prior to reduction reactions.

RESULTS :

The ESI source as an electrolysis cell : Study of a series of compounds with different redox potentials.

We checked a series of compounds in pure CH_2Cl_2 without any previous electrochemical treatment. The corresponding m/z peaks observed for each compound studied are presented in table 1.

Compound	Molecular Weight	Redox potential V / SCE	Peak observed	Process (Mode)
Ferrocene	186.0	+0.38	$M^{\bullet +}$	Oxidation (Positive)
β-Carotene	536.9	+0.53 (ref 14)	$M^{\bullet +}$	
Acetyl Ferrocene	228.1	+0.62	$M^{\bullet +}$	
ZnTPP	678.1	+0.77	$M^{\bullet +}$	
H2TPP	614.7	+1.01	$[M+H]^+$	
Fullerene C_{60} -C_{70}	720.7	-	Non Detected	
DDQ	227.0	+0.62	$M^{\bullet -}$	Reduction (Negative)
TCNQ	204.2	+0.15	$M^{\bullet -}$	
Benzoquinone	108.1	-0.57	$M^{\bullet -}$	
Fullerene C_{60}-C_{70}	720.7 - 840.8	-0.60	$M^{\bullet -}$	
ZnTPP	678.1	-1.35 *	Non Detected	

H2TPP : tetraphenyl porphyrin, ZnTPP : zinc tetraphenyl porphyrin, TCNQ : tetracyanoquinodimethane, DDQ : dichlorodicyanoquinone.
The redox potentials were measured in CH_2Cl_2+0.1M $TBAPF_6$ by cyclic voltammetry (scan rate 0.1V/s) on a Pt electrode.
* Value determined in DMF/$TBAPF_6$

Table 1 : Summary of results of redox processes in the ES source.

In each case the molecular ions are obtained and the isotopic pattern of the radical ion corresponds exactly to the expected one, showing that these ions are singly charged and that the ionization did not involve protonation (except for H_2TPP) or other cation addition (see figure 1 in the case of fullerite).

The value of the redox potential is an essential factor : a compound having a redox potential within -0.8 and +1.0V /SCE in pure CH_2Cl_2 should be analysable by ESI-MS without any previous electrochemical treatment. As expected, the intensity of the molecular ion is correlated to the concentration of the sample ranging from 10^{-5} M to 10^{-3} M and in the case of the least oxidizable or reducible compounds, the samples have to be concentrated. It is important to note, that in the case of reduction reactions, the solution has to be intensively degassed to remove oxygen from the sample. Indeed, oxygen may be reduced before the analyte, thus preventing its detection. The problem arises mainly for redox potentials below -0.8V (/SCE). This may explain why ZnTPP could not be detected in the negative mode.

Study of fullerite :

Cyclic voltammetry showed the existence of three reduction potentials for the fullerite: -0.60V, -1.00V and -1.45V /SCE, and Allemand *et al.*[11] have shown that both C_{60} and C_{70} have the same first reduction potential. The ESI-mass spectrum, in the negative mode, of the fullerite (figure 1), without any previous electrolysis showed two peaks corresponding to the monoreduced species $M^{\cdot-}$ for C_{60} and for C_{70}. Their relative intensities correspond to the proportions of C_{60} and C_{70} initially present in the fullerite, which indicates that the method is not discriminating. As mentioned before, the total removal of oxygen is essential.

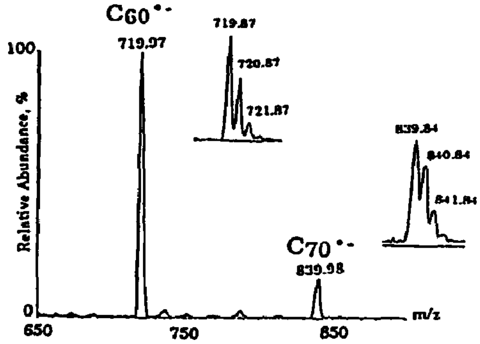

Figure 1 : ESI-mass spectrum of fullerite without any previous electrolysis.

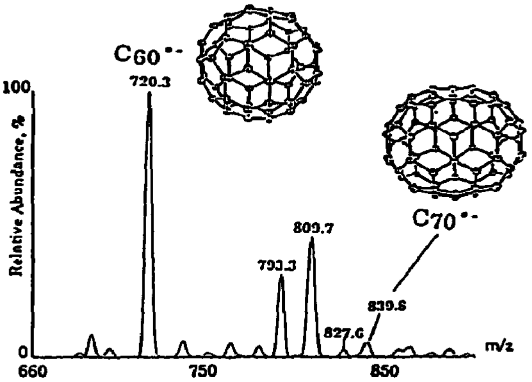

Figure 2 : ESI-mass spectrum of fullerite after potentiostatic bulk electrolysis (at -0.70 V/SCE)

Two modes of electrolysis of the fullerite have been investigated : potentiostatic reduction and intensiostatic reduction. The ESI-MS spectra of the fullerite obtained after potentiostatic reduction are not reproducible, and show several uninterpreted peaks besides those corresponding to $M^{\cdot-}$ for C_{60} and C_{70} (figure 2). The relative intensities of C_{60} and C_{70} do not correspond to the proportions of C_{60} and C_{70} in fullerite and are even different from one experiment to another. The study has been performed for the first and the second reduction step and show the same uncharacterized peaks. However, two peaks at m/z=708 and m/z=828 have been observed several times at the second reduction stage after potentiostatic reduction, and they have been attributed to the doubly-charged molecular anions of higher fullerenes. Indeed Yeretzian *et al.*[12] described coalescence reactions of fullerenes: twice C_{60} give one C_{118} and twice C_{70} give one C_{138}. The mechanism of formation of these peaks has not been explained yet, and they are found not to be reproducible.

In the case of intensiostatic reduction, optimal conditions have been defined in order to obtain a good image of the species present in the solution: short time of electrolysis (10 minutes), medium accelerating potentials (Vc^{3b}= 50V). Only in this case are the ESI-MS spectra comparable to those obtained without any previous reduction, except the presence of peaks corresponding to the supporting electrolyte. In both cases, the presence of TBA-PF_6 as supporting electrolyte at a concentration of 10^{-2} M disturbs the spray, entailing a loss of intensity, as any concentrated salt does[13].

CONCLUSION :

The study of neutral species, dissolved in pure solvent, by ESI-MS in the positive and negative mode, without any previous electrolysis is possible, provided that the redox potential of the compound studied lies within the following potentials limits: -0.8 and +1.0V /SCE. Under these conditions the ESI source behaves like an electrolysis cell with many advantages compared to off-line electrolysis: no suppporting electrolyte, no side-reactions of the generated charged species because of the shorter time involved. Further studies are under way to extend the limits of the available redox potential range.

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