

Radical Ion Generation Processes of Organic Compounds in Electrospray Ionization Mass Spectrometry

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Abstract: In electrospray ionization mass spectrometry (ESI-MS), charge formation is proposed to occur as a result of acid-base reactions or a coordination with metal cations. However, the increasing number of papers have reported the formation of radical ions (molecular ions) as a result of loss of one or two electrons. In this paper, we present an overview of the current literature on the balance between the formation of molecular ions and of protonated forms of organic compounds in ESI-MS. Correlations between the radical formation process and the ionization energy, calculated by molecular orbital methods or obtained by electrochemical techniques, were applied to explain this apparently unexpected behavior. The use of high level theoretical methods especially designed to produce accurate thermochemical data is also reported.

Keywords: Electrospray ionization, mass spectrometry, organic compounds, radical ion generation, molecular ion, computational thermochemistry.

1. INTRODUCTION

Mass spectrometry (MS) is a powerful analytical tool for identifying unknown molecular structure and for probing the fundamental principles of chemistry [1]. The technique has been extensively employed for the analysis of a diversity of organic compounds, including natural products [2-7], synthetic intermediates [8,9], pharmaceuticals [10,11], synthetic polymers [12], peptides [13-16], and proteins [17-19]. In an MS analysis, gaseous ions of the sample are generated and analyzed (separated) according to their m/z (mass-to-charge ratio). The sample ionization (e. g., the generation of charged particles from neutral species [20]) can be achieved by using different ionization methods, chosen according to the characteristics of the sample such as molecular weight and thermolability [21].

Electrospray ionization mass spectrometry (ESI-MS) has emerged as a particularly useful technique for the analysis of thermolabile and high-molecular weight compounds [21]. It has been proposed in the literature that the ionization process in ESI-MS occurs as the result of acid-base reactions (protonation/deprotonation) and/or a coordination with metal cations, thus giving rise to protonated molecules ($[M+H]^+$), deprotonated molecules ($[M-H]^-$) and cationized molecules (i. e., $[M+Na]^+$) [21,22]. However, an increasing number of papers have been recently published reporting the formation of radical ions (molecular ions) as a result of loss of one or two electrons.

In this paper, we present an overview of the current literature on the balance between the formation of molecular ions and protonated forms of organic compounds in electrospray ionization mass spectrometry (ESI-MS). We also provide an overview of the electronic structure methods and electrochemical techniques that can be employed as tools for understanding this apparently unexpected behavior.

2. THE ESI SOURCE AS AN ELECTROCHEMICAL CELL

Mechanisms of Gas-Phase Ion Generation in ESI

In electrospray ionization (ESI), the sample is dissolved in a polar, volatile solvent and pumped through a narrow stainless steel capillary (75–150 μm i.d.) at a flow rate in the range of 1 $\mu\text{L min}^{-1}$ and 1 mL min^{-1} [23]. A sufficiently high voltage (typically 1-4 kV) is applied at the capillary tip, which is situated within the ionization source of the mass spectrometer [24-26]. This high voltage causes a charge migration of species in solution to the capillary metal/solution interface, resulting in an electric double layer [27]. Thus, limitations on charge formation common to other ionization techniques (i.e., electron ionization) are minimized in ESI because the pre-organization of charges occurs in solution [27].

In the capillary tip, the electrostatic forces on the pre-formed ions in the electric double layer counterbalance the surface tension, resulting in a conical surface called a Taylor cone [28]. The increase in the voltage destabilizes the Taylor cone and the sample emerging from the capillary tip is dispersed into a very fine spray of highly charged droplets, a process that is aided by a co-axially introduced nebulizing

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gas (usually nitrogen) flowing around the outside of the capillary. The charged droplets diminish in size due to solvent evaporation, assisted by a flow of warm nitrogen, known as drying gas that passes across the front of the ionization source.

The solvent evaporation leads to an increase in the concentration of the formal charge q at the droplet surface until the Rayleigh limit is reached [28]. At the Rayleigh limit, Coulombic repulsion overcomes the droplet surface tension and the droplet explodes. This 'Coulombic explosion' forms a series of smaller droplets of lower charge [29]. The process of solvent evaporation followed by droplet explosion is repeated until individually charged analyte ions free of solvent are formed. This results in the formation of a 'spray' of charged species [29-31], some of which pass through a sampling cone or orifice into an intermediate vacuum region and, from there, through a small aperture into the analyzer of the mass spectrometer, maintained under high vacuum. The charges are statistically distributed among the available charge sites of the analyte, permitting the formation of multiply charged ions under the correct conditions [32].

Two mechanisms have been proposed for gaseous ions formation in ESI, both occurring during the droplet desolvation [33]. The first model, known as 'charge residual model' (CRM) proposes that successive droplet fissions occur until individual ions are formed in the gas-phase [34]. The second model, known as the 'ion evaporation model' (IEM) assumes that the ions are expelled from the droplets to the gas-phase before total droplet fissions. These fissions have been shown to occur when the droplet radius becomes smaller than 10 nm [35]. Regardless of the mechanism by which the gas-phase ions are produced by ESI, they can be analyzed for their mass-to-charge ratio within the mass spectrometer [23].

Depending on the voltage applied to the metal capillary, positive ions (positive mode) or negative ions (negative mode) can be analyzed in ESI-MS [35]. The selected mode must take into account mainly the analyte characteristics and the purpose of the analysis. In the positive mode of analysis, ESI produces ions as a result of acid-base reactions (protonation) or coordination with metal cations (usually Na^+ or K^+). In this case, protonated molecules ($[\text{M}+\text{H}]^+$) and/or cationized molecules (i.e., $[\text{M}+\text{Na}]^+$) are commonly predominant in the ESI-MS spectra. However, the possibility of generating radical ions $[\text{M}]^{+\bullet}$ (molecular ions) using ESI-MS in the positive ion mode of analysis has recently been reported to be an important alternative for the analysis of organic compounds with low oxidation potentials, such as polycyclic aromatic hydrocarbons (PAH) [36]. In order to understand the apparently unexpected formation of radical ions, the ESI source has been compared to an electrolytic cell [37]. Thus, some basic concepts regarding the ESI source as an electrolytic cell are presented below.

Redox Reactions in the ESI Source

From the electrochemical point of view, the ES ion source has been characterized as a controlled-current electrolytic (CCE) flow cell [36,37]. The metal capillary

(usually stainless steel) in contact with the solution placed at or upstream of the ES point in which the charged ESI droplet plume is generated (i.e., the ES emitter). This is the working electrode since the reactions of analytical significance in this system occur on it. The second electrode, or counter electrode, is the atmospheric sampling aperture plate or inlet capillary and the various lens elements and detector of the mass spectrometer [37, 38]. The third component of the CCE flow cell is the average current that flows in the cell, i.e., the ES current, which is the gaseous ion flow that is sprayed toward the counter electrode due to the electric field.

Although the ES ion source exhibits the three components of an electrolytic cell, it has been established that it behaves as a CCE flow cell only under appropriate operation conditions [38]. In these cases, electron transfer reactions (redox reactions) between electrode and solution can occur at the conductive contact to the solution at the spray end of the ES device. Thus, depending on the flow rate of the sample within the capillary, the magnitude of the ES current and the oxidation potential of the analyte, electrochemical processes can have a significant effect on the identity and relative abundances of the ions in an ESI mass spectrum [38].

The magnitude of the electron transfer (redox reactions) at the capillary tip and the charge separation at the droplet surfaces depend on the level of organization of the electric double layer that is formed at the capillary/solution interface [29]. Redox reactions are more favored at low flow rates, since there is an increase in the time during which neutral species are in contact with the capillary/solution interface [37-40].

The magnitude of the ES current is related to the rate of the charge separation process (the droplet generation rate times the average charge per droplet). This process is determined by several parameters, including the electric field applied between the electrodes, the solution flow-rate and the solution conductivity [38], as evidenced by experimental results and the following semi-empirical relationship (Equation 1) [41-45].

$$I_{\text{ES}} = [(4\pi/\epsilon)^3 (9\gamma)^2 \epsilon_0^5] (\kappa E)^{3/7} v_f^{4/7} \quad (\text{Equation 1})$$

where ϵ and ϵ_0 are the permittivity of the solvent and vacuum, respectively; γ is the surface tension of the solvent; κ is the solution conductivity, E is the imposed electric field at the capillary and v_f is the flow rate.

Blade and co-workers used a zinc capillary to analyze solutions containing different concentrations of Zn^{+2} [27]. They detected Zn^{+2} ions in the sprayed solutions and established that oxidation reactions could occur when the ES current is of same magnitude as the Faraday current (i_F) and the potential difference between the capillary and the counter electrode is equal to or higher than the oxidation potential of the species in solution (Equation 2) [29, 37, 38].

$$I_{\text{ES}} = i_F = \sum n_j A_j F v_f \quad (\text{Equation 2})$$

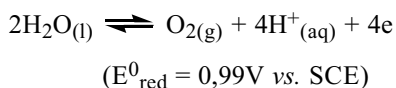
where n is the number of electrons; A is the surface area of the electrode, F is the Faraday constant and v_f is the flow rate.

The influence of the oxidation potential on the relative abundance of radical ions in the ESI-MS spectrum was investigated by Vandell and co-workers [46]. They observed that the most abundant ions in the ESI-MS spectra of equimolar mixtures of metalloporphyrins result from oxidation reactions, which depend on the redox potential. Van Berkel and Zhou demonstrated that the increase in the conductivity of the solution upon addition of electrolytes (such as lithium triflate) assists in the oxidation reactions [37]. Conversely, Dupont and co-workers reported that electrolyte addition to the solution destabilized the radical ions of C₆₀ [47].

The capability of a neutral organic molecule to lose one electron (i.e., oxidize) depends on the energy of the highest-occupied molecular orbital (HOMO). This energy can be estimated by theoretical calculations, from experimental measurements of the oxidation potential of the analyte (E_{ox}) or from its ionization energy (IE) in the gas-phase. Although a correlation between E_{ox} and IE has been demonstrated for a large number of compounds, these values are affected by solvation and must therefore be measured under same experimental conditions, in the same solvent system [48].

Van Berkel and co-workers have extensively investigated the electrochemical processes in the ES source. They demonstrated the formation of oxidized/reduced species using UV/visible and MS and confirmed the correlation between the spray current and the solution conductivity [27, 49-52]. The authors also reported that the minimum voltage required to oxidize the analyte increases linearly with its redox potential.

Despite the fact that the ES source can behave as a CCE flow cell under well-defined conditions, thus generating radical ions (molecular ions) by oxidizing to ($M^{+\bullet}$) or reducing to (M^{\bullet}) neutral analyte molecules, it has been demonstrated that the proton concentration that results in the formation of protonated molecules ($[M+H]^+$) also increases under the same conditions [53]. Indeed, Van Berkel and co-workers reported that the pH in unbuffered systems decreased four units due to water oxidation, as shown below (where SCE means saturated calomel electrode):



Thus, the conditions that make it possible for the ES source to behave as a CCE flow cell are clearly necessary, but not sufficient for understanding the balance between redox and acid-base (protonation/deprotonation) reactions that lead to the formation of either radical ions (molecular ions of $M^{+\bullet}$ or M^{\bullet} type) or protonated/deprotonated molecules ($[M+H]^+$ or $[M-H]^-$), respectively, and their relative abundances in the ESI-MS spectrum. For this purpose, thermochemical properties such as proton affinity and/or the ionization energy of the analyte, solvent system or even contaminants have been successfully used [54].

For compounds that exhibit high proton affinity and basic functionalities that can serve as possible protonation sites, such as biomolecules [55], protonation has been reported to be more favored than electrochemical oxidation

[56]. In contrast, compounds that exhibit low oxidation potentials and structural features that stabilize the unpaired electron (e.g., a long conjugated π system) are commonly oxidized in the ESI positive ion mode, thus generating positive radical ions ($M^{+\bullet}$). Similarly, negative radical ions (M^{\bullet}) may be generated in the ESI negative ion mode from molecules exhibiting low reduction potentials and a long conjugated π system.

3. RADICAL IONS OF SOME CLASSES OF ORGANIC COMPOUNDS

Polycyclic Aromatic Hydrocarbons (PAH)

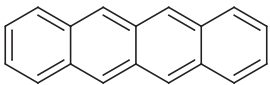
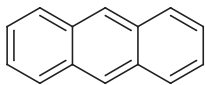
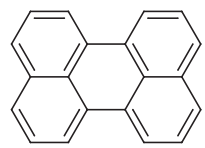
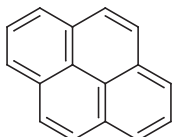
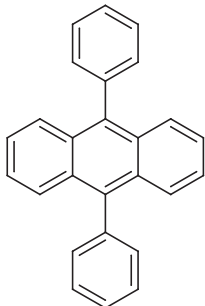
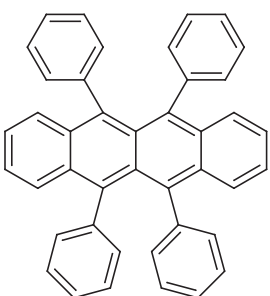
Polycyclic aromatic hydrocarbons (PAH) are a class of compounds produced as result of incomplete combustion of organic matter [57]. Studies undertaken on various environmental matrices, such as effluents of coal combustion, vehicle exhaust, lubricant oils and cigarette smoke have demonstrated that the PAH present in these mixtures are the major contributors to their toxicity [58]. Furthermore, a number of papers have dealt with the deleterious effects of PAH on the human body, including cancer [59].

The formation of positive radical ions ($M^{+\bullet}$) in ESI-MS has been reported for a series of PAH (Table 1 shows several examples) [36,50]. The results are very similar to those described by Phelps and Marcoux for the electrochemical generation of radical ions from PAH [60]. Van Berkel, McLuckey and Glish investigated the behavior of benzantracene (**1**), anthracene (**2**), perylene (**3**) and other PAH in an ESI source [50]. The authors demonstrated that positive radical ions can be easily generated when the PAH exhibits $E_{1/2\text{ox}}$ lower than 1.25 V and an ionization energy (IE) below 7.45 eV. For naphthalene, however, neither the molecular ion ($M^{+\bullet}$) nor protonated molecules ($[M+H]^+$) could be detected in the ESI-MS spectrum. This fact was attributed to the EI (8.14 eV) and E_{ox} (1.65 V vs. SCE) values of naphthalene, which were significantly higher than those of other PAH, as well as its lower proton affinity (802.9 kJ mol⁻¹) values in comparison to other PAH.

Recently, Laali and co-workers studied the complexation of Ag⁺ ions with substituted PAH [61]. In order to understand the structure of the cationized molecule ($[M+Ag]^+$), the authors compared the experimental results to those obtained by electronic structure calculations. The absence of the molecular ions ($M^{+\bullet}$) was demonstrated to be either due to substituent effects on the stabilization of Ag⁺/PAH complexes or even due to the stability of the positive radical ion. The authors proposed that electron-donor groups assist in the stabilization of the Ag⁺/PAH complex whereas electron-acceptor groups destabilize the positive radical ions [61].

For PAH with oxidation potentials lower than 1.0 V (vs. SCE), the formation of radical ions was easily observed [52]. In these cases, molecular ions ($M^{+\bullet}$) were predominant in the ESI-MS spectrum, whereas the protonated molecule ($[M+H]^+$) had a very long low relative abundance. Studies with heterocyclic aromatic compounds also can produce radical ions through ESI ionization (Table 2).

Table 1. Structures of Some Polycyclic Aromatic Hydrocarbons (PAH)

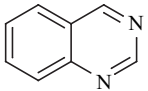
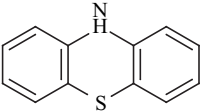
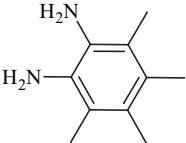
Structure	Formula/Nominal Masses	Species	Eox (V vs. SCE)	Ref.
 2,3-benzanthracene (1)	C ₁₈ H ₁₀ 202	M ⁺ •	0.98	80
 anthracene (2)	C ₁₄ H ₁₀ 178	M ⁺ •	1.19	80
 perylene (3)	C ₂₀ H ₁₂ 252	M ⁺ •	1.04	80
 pyrene (4)	C ₁₆ H ₁₀ 202	M ⁺ •	1.25	80
 9,10 diphenylanthracene (5)	C ₂₆ H ₁₈ 330	M ⁺ •	1.22	80
 rubrene (6)	C ₄₂ H ₂₈ 532	M ⁺ •	0.82	80

Polyenes

Carotenoids are pigments derived from isoprene units and are widely distributed in bacteria, fungi, algae and plants, where they play important roles. This class of compounds comprise of carotenes and xanthophylls, which are oxygen-containing carotenes (Table 3). Although animals

do not synthesize carotenoids, they are nutritionally acquired and are responsible for several biological roles, such as antioxidants, single oxygen quenchers, vitamin A (Table 3; **11**, **12**), precursors and membrane stabilizers [62-66]. Their remarkable antioxidant activity is due to the presence of an extended polyene conjugation [63]. Moreover, carotenoids

Table 2. Heterocycle Aromatic and Aromatic Amines

Structure	Formula/Nominal Masses	Species	E _{ox} (V vs. SCE)	Ref.
 isoquinoline (7)	$C_8H_6N_2$ 130	$M^{+\bullet}$	8.53*	**
 phenothiazine (8)	$C_{12}H_9NS$ 199	$M^{+\bullet}$; $[M+H]^+$	0.56	80
 tetramethylphenylenediamine (TMPD) (9)	$C_{10}H_{16}N_2$ 164	$M^{+\bullet}$	1.02	80

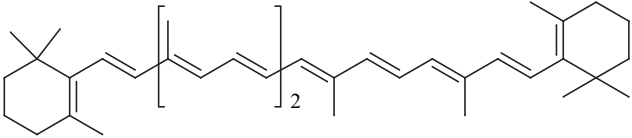
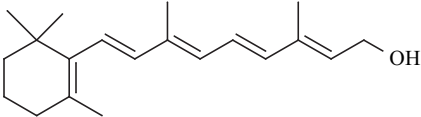
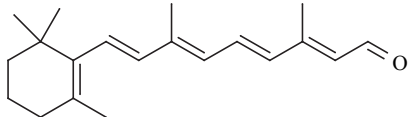
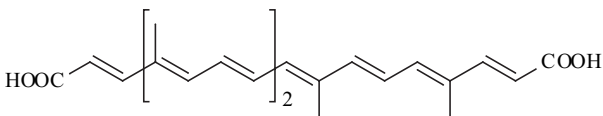
* Ionization energy (eV obtained at webbook.nist.gov/chemistry)

** Data not published – principal author

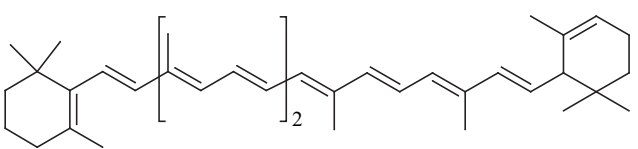
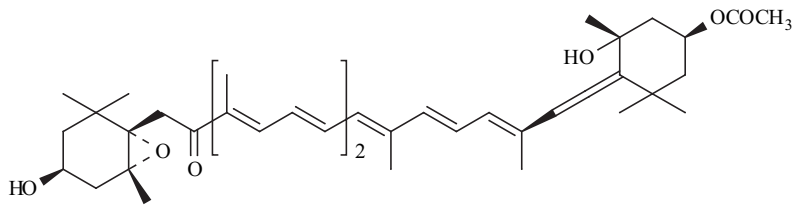
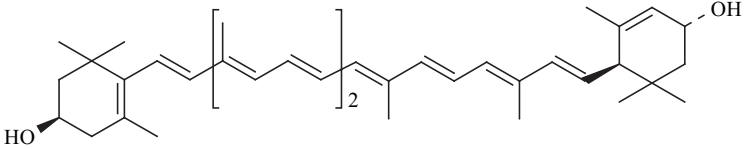
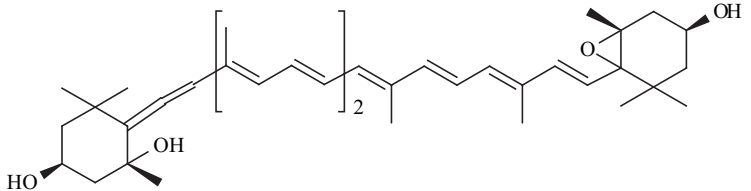
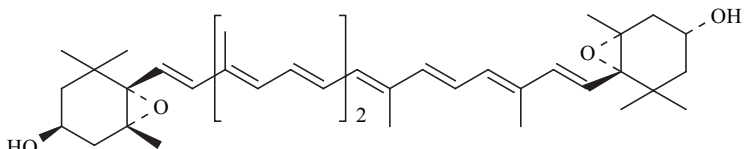
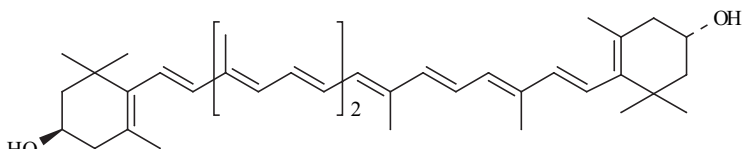
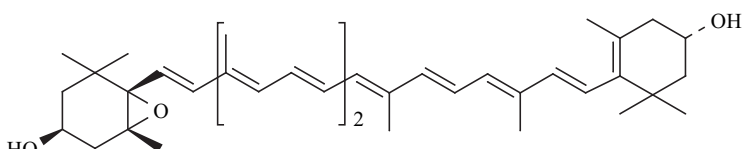
interact with biological membranes according to their structure and polarity [67].

In the biosynthetic route, modifications such as cyclization, substitution, elimination, addition and

Table 3. Structures of Polyenes, Carotenoids and Xantophylls

Structure	Formula/Nominal Masses	Species Observed	Ref.
 β-carotene (10)	$C_{40}H_{56}$ 536	$M^{+\bullet}$, M^{2+}	70,71
 retinol (11)	$C_{20}H_{30}O$ 286	$[M+H]^+$	71
 retinal (12)	$C_{20}H_{28}O$ 284	$[M+H]^+$, $M^{+\bullet}$	71
 bixin (13)	$C_{24}H_{28}O_4$ 380	$M^{+\bullet}$	71

(Table 3)contd.....

Structure	Formula/Nominal Masses	Species Observed	Ref.
 <p style="text-align: center;">α-carotene (14)</p>	C ₄₀ H ₅₆ 536	M ^{+•} , M ²⁺	70,73
 <p style="text-align: center;">fucoxanthin (15)</p>	C ₄₂ H ₅₈ O ₆ 658	[M+H] ⁺ , M ^{+•}	72
 <p style="text-align: center;">lutein (16)</p>	C ₄₀ H ₅₆ O ₂ 568	[M+H] ⁺ , M ^{+•}	72
 <p style="text-align: center;">neoxanthin (17)</p>	C ₄₀ H ₅₆ O ₄ 600	[M+H] ⁺ , M ^{+•}	72
 <p style="text-align: center;">violaxanthin (18)</p>	C ₄₀ H ₅₆ O ₄ 600	[M+H] ⁺ , M ^{+•}	72
 <p style="text-align: center;">zeaxanthin (19)</p>	C ₄₀ H ₅₆ O ₂ 568	[M+H] ⁺ , M ^{+•}	72
 <p style="text-align: center;">anteraxanthin (20)</p>	C ₄₀ H ₅₆ O ₃ 584	[M+H] ⁺ , M ^{+•}	72

rearrangements result in a diversity of colorful and useful structures. Lycopene, the basic skeleton of carotenoids and xanthophylls, can give rise to either α - or β -carotene (Table 3;

10, 14). Hydroxylation of α -carotene (14) yields lutein (16), while zeaxanthin (19) results from the hydroxylation of β -carotene. According to their biosynthesis, one or two

consecutive epoxidations of zeaxanthin result in either antheraxanthin (**20**) or violaxanthin (**18**), respectively. Further rearrangement of the violaxanthin structure results in the formation of neoxanthin (**17**) [68]. In this regard, more than 600 different carotenoid structures are currently known [69]. Due to an assumed favorable correlation between a high carotenoid intake and health benefits, besides their physiological and ecological importance, many techniques have been developed for analyzing this class of compounds, including ESI-MS.

ESI-MS studies on carotenoids were initially performed using carotenes [70] and subsequently extended to heteroatom-containing polyenes (xanthophylls) [71,72]. The relatively rapid formation of doubly charged radical ions of α -carotene and β -carotene in pure aprotic solvents was demonstrated by Van Berkel and Zhou [73]. These ions were also observed when oxidizing agents (such as 0.1 mM lithium triflate) were added to the solution to optimize the charge transfer [73]. These doubly charged ions were formed because of the proximity between their oxidation potential values ($E_{\text{ox}} = 0.8\text{V}$ e $E_{\text{ox}} = 1.1\text{V}$ vs. $\text{Ag}/\text{AgCl}_{\text{sat}}$) [71]. In addition, Van Breemen reported that radical ions were the only species observed in LC-MS analyses of the carotenoids mentioned above and of three xanthophylls, using heptafluoro-butanol as solvent [70]. However, doubly charged ions were not observed under these conditions.

The generation of doubly charged radical ions of β -carotenes using ESI-MS in the absence of oxidizing agents in solution and/or in supporting electrolyte, which could in principle result in signal suppression [74], was confirmed by Guaratini and co-workers [71,72]. On the basis of E_{ox} and proton affinity values obtained by cyclic voltammetry and semi-empirical theoretical methods, respectively, the authors obtained a correlation between the oxidation potential and radical ion generation similar to that of PAH. They proposed that formation of these radical ions follows a mechanism similar to those previously reported for PAH [36,50]. The formation of the $\text{M}^{+\bullet}$ species was also observed in HR-MALDI-MS. The same authors subsequently extended their studies to other conjugated compounds, such as xanthophylls. In most cases, both the radical ion ($\text{M}^{+\bullet}$) and protonated molecules ($[\text{M}+\text{H}]^+$) were observed in the ESI-MS spectra of xanthophylls when 5% (v/v) acetic acid was added to the solvent system. These results confirmed that the solvent system can play a key role in the balance between protonated molecules and positive radical ions [72].

Metalloenes

The field of organometallic compounds was strongly stimulated by the discovery of *bis*(cyclopentadienyl)iron derivatives, such as ferrocene [75]. Since then, cyclopentadienyl ligands have played a key role in the

Table 4. Structure of Metalloporphyrins

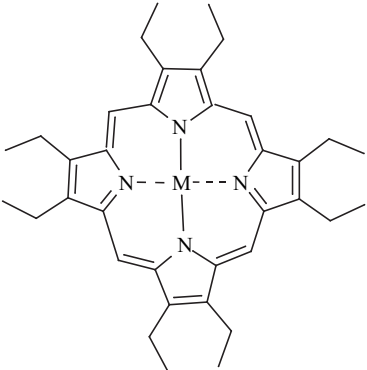
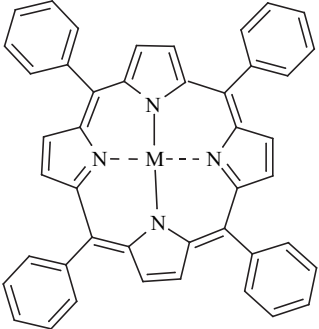

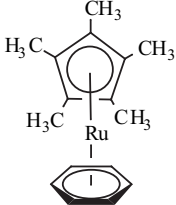
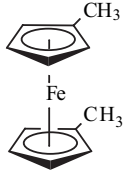
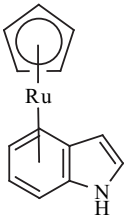
Structure	Metal	Nominal Masses	$E_{1/2}$	Species	Ref.
 octaethylporphyrin (21)	Zn	596	0.745	$\text{M}^{+\bullet}$	46
	Ni	590	0.860	$\text{M}^{+\bullet}$	46,73
	Mg	556	0.545	$\text{M}^{+\bullet}$	46
	Cu	595	0.840	$\text{M}^{+\bullet}$	46
	VaO	599/560	1.030	$\text{M}^{+\bullet}; [\text{M}+\text{H}]^+$	46
 tetraphenylporphyrin (22)	Mg	636	1.05	$\text{M}^{+\bullet}$	46
	Ni	670	1.09	$\text{M}^{+\bullet}$	46,73
	Cu	675	1.07	$\text{M}^{+\bullet}$	46
	Zn	676	0.87	$\text{M}^{+\bullet}$	46,47
	VaO	679	1.22	$\text{M}^{+\bullet}$	46

Table 5. Structure of Metallocenes

Structure	Metal	Formula/Nominal Masses	Species	Ref.
 (23)	Ru (ruthenocene)	C ₁₀ H ₂₀ Ru / 232	M ^{+•}	40
	Fe(ferrocene)	C ₁₀ H ₂₀ Fe / 186	M ^{+•}	40
	Os (osmocene)	C ₁₀ H ₂₀ Os / 322	[M+H] ⁺	40
 (pentamethylruthenocene)(24)	Ru	C ₁₅ H ₂₆ Fe / 260	M ^{+•}	40
 1,1'-dimethylferrocene (25)	Fe	(CH ₃ C ₅ H ₄) ₂ Fe / 186	M ^{+•}	40
 (26)	Ru	C ₁₃ H ₁₂ NFe / 237	M ^{+•}	40

discovery of new organometallic compounds. Before the 1990s, most of the organometallic compounds synthesized had either cyclopentadienyl or a derivative thereof in their structures [76].

Due to their low ionization energies, metallocenes can be characterized by conventional electrochemical techniques [77]. However, since 1995, mass spectrometry employing electron ionization (EI-MS), photoionization (PI-MS), chemical ionization (CI-MS) and fast atom bombardment (FAB-MS) has been used to analyze a number of metallocenes [78,79]. Xu and co-workers were the first to report the generation of radical ions of metallocenes by electrochemical oxidation using ESI-MS [80]. They also reported the occurrence of nucleophilic addition reactions during the ESI-MS analyses, thus revealing the great potential of the technique for studies on this class of compounds (Table 4 and 5).

ESI-MS studies of other organometallics, such as porphyrins (21, 22) were performed by Vandell, Patrick and

Limbach [46]. These studies revealed that the relative intensity of each ion in the ESI-MS spectrum is directly dependent on the solution concentration and the oxidation potential of each compound. For equimolecular mixtures, the most intense positive radical ions were observed for those compounds exhibiting the lowest oxidation potential. These results are in concordance with those reported by Van Berkel and Zhou for the characterization of the ESI source as a CCE flow cell [37]. In this case, however, the analyses were performed in the absence of supporting electrolytes and/or other agents to optimize the charge transfer.

ESI-MS studies using Ni^{II} octaethylporphyrins (OEP) demonstrated the effect of the supporting electrolyte on the solution conductivity [81]. In the experiments, the Ni^{II} OEP ($E_{1/2ox} = 0.73V$ and $IE = 6.38eV$) and the flow rate were maintained constant. An increase in solution conductivity led to a concomitant increase in the concentration of the oxidized species. These results were confirmed by the use of an UV/vis detector coupled to the ESI source. Thus, the

magnitude of i_{ES} could be readily modulated by varying the solution conductivity with added supporting electrolyte, which facilitates the charge transfer and the formation of reduced or oxidized species. Studies of Mg^{2+} , Cu^{2+} and Zn^{2+} complexes of OEP by ESI-MS also demonstrated the formation of $M^{+\bullet}$ ions. In these cases, it was possible to establish a correlation between the low oxidation potentials of these metallic cations (0.54, 0.79 and 0.63V vs. SCE, respectively) with the cation radical formation in the ES source [82]. In addition, the formation of doubly charged positive radical ions of Ni^{2+} and Co^{2+} OEP by two sequential one-electron oxidations was reported to be possible due to the addition of supporting electrolytes to the solution and a low flow rate [73].

The fact that all of the metaloporphyrins and ferrocenes (Table 5) studied were oxidized clearly reinforces the electrolytic nature of electrospray ionization [80].

Quinones

Quinones are a class of natural products that occur in several living organisms. They exert essential function in the electron carrier chain and are also mediators of vital processes such as respiration and photosynthesis [83]. Knowledge of the thermochemical properties of quinone derivatives has assisted in understanding their anti-neoplastic, trypanocidal and molluscocidal activities [84].

The formation of negative radical ions ($M^{\bullet-}$) of substituted quinones was described in ESI-MS using the negative ion mode of analysis (Table 6) [85]. This was attributed to the low reduction potential of these compounds

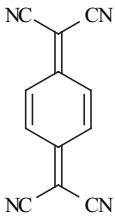
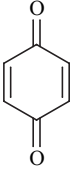
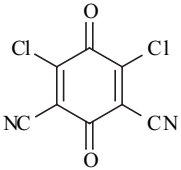
and the stabilization of the unpaired electron by resonance. The correlation between the formation of negative radical ions ($M^{\bullet-}$) and a low reduction potential was also reported for tetracyanoquinone (27), benzoquinone (28) and dichlorodicyanobenzoquinone (29) [86].

Fullerenes

The electrochemical behavior of fullerenes and their derivatives has attracted the interest of various research groups. Studies performed in different solvent systems demonstrated that negative radical ions of C_{60} can be obtained by electrochemical reduction at a relatively positive potential [87,88]. Hettich and co-workers suggested that the electron affinity of fluorinated fullerenes is higher than that of their non-fluorinated analogs by 1.5 eV. The authors also established that doubly charged negative radical ions can be formed in the gas-phase by means of sequential electron capture and further investigated by mass spectrometry [89]. This stimulated a study of $C_{60}F_{48}$ by cyclic voltammetry and by ESI-MS characterization of the products that are formed as a result of successive additions of electrons in solution using different electron donors [90]. Cyclic voltammetry experiments showed that the formation of the negative radical ion ($M^{\bullet-}$) of $C_{60}F_{48}$ in CH_2Cl_2 occurred at 0.79 V vs. SCE and is reversible reduction.

Dupont and co-workers investigated the potential of an ES source for generating radical ions from neutral analytes with no previous electrolysis [47]. Their ESI-MS results were confirmed by use of off-line electrochemical analyses. The authors also reported the reduction of O_2 in solution by

Table 6. Structures of Quinones

Structure	Formula/Nominal Masses	Species	E_{ox} (V vs. SCE)	Ref.
 <p>tetracyanoquinodimethane (27)</p>	$C_{12}H_4N_4$ 204	$M^{\bullet-}$	0.15	47
 <p>Benzoquinone (28)</p>	$C_6H_4O_2$	$M^{\bullet-}$	-0.57	47
 <p>dichlorodicyanobenzoquinone (29)</p>	$C_6O_2CN_2Cl_2$ 227	$M^{\bullet-}$	0.62	78

compounds exhibiting E_{red} lower than -0.8V (vs. SCE). The results obtained from ESI-MS studies in CH_2Cl_2 demonstrated that neutral organic species, dissolved in organic solvents of high purity, can form radical ions in the interval from -0.8 to 1.0V (vs. SCE). The main advantage was that a supporting electrolyte, which could cause oscillations in the spray generation, was not required [27].

4. THE USE OF COMPUTATIONAL CHEMISTRY IN THE STUDY OF SPECIES IONIZED BY ESI

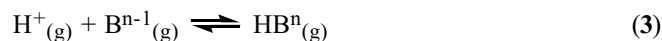
In ESI, most organic compounds can be ionized by protonation/desprotonation (Brønsted-Lowry acid/base reactions) and/or by coordination with ions present in the sample solution [91-93]. The basic sites of molecules can accommodate a proton or other metal cations, while the anions are usually produced by the loss of acidic hydrogens. The direct formation of radical ions in ESI-MS is of significance only in some cases, as previously discussed in this review.

Although the ionization processes occur initially in solution, the identity of the gaseous ions is related to their thermochemical properties in the gas-phase. Values of these properties can be obtained experimentally or estimated by theoretical computational methods. Obtaining thermochemical data from experiment is often difficult due to a number of limitations (e.g., the thermolability of the molecule and its subsequent degradation, as occurs in calorimetry).

One of the most useful experimental methods is the kinetic method, developed by Cooks and co-workers [94], which has suffered minor alterations over the years. The kinetic (or thermokinetic) method is an important tool for analyzing biological molecules, especially aminoacids and proteins [95]. This method is based on the formation of a dimeric conjugated or complex between the target molecule and a molecule with pre-defined thermochemical values. One of the limitations of this method is the number of affinity sites [96]. Another difficulty is the difference between the entropies of the two ions that are formed. Due to these limitations, theoretical computational calculations provide an attractive alternative for the determination of thermochemical quantities. Furthermore, given the advances in computational chemical models, the theoretical results are much closed to the experimental ones (often within 0.5 kcal mol^{-1}) [97].

In recent years, computational thermochemistry has been shown to be a powerful and useful alternative for obtaining proton affinity (PA) and basicity (BG) values of organic compounds in the gas phase [90-93, 98-100]. The increasing interest in the determination of these thermochemical quantities is not surprising due to the widespread occurrence of proton transfer in organic and biochemical reactions [101]. Furthermore, the protonated molecule is a common intermediate in the global ion-forming process in ESI-MS [93,102,103]. Thus, a knowledge of the intrinsic basicity of a compound in the gas-phase ($-\Delta G_f$) plays a key role in understanding its reactivity. The great advantage of computational calculations of the proton affinity is the fact that these values can be estimated both relatively and absolutely [92].

Equation 3 can be assumed for all of the reactions involving bond heterolysis.



The negative of the standard free energy charge ($-\Delta G^\circ$) for this reaction is the basicity of B^{n-1} (BG_1), whereas the negative of the standard enthalpy charge ($-\Delta H^\circ$) is the proton affinity (PA) of B^{n-1} .

Although calculations using heterolytic reactions can give satisfactory results, the use of isodesmic reactions, which not only keep constant the number and type of bonds but also the bond character, provides more consistently reliable results for heat of formation in the gas-phase [104,105]. Homodesmotic reactions, a subclass of isodesmic reactions, in which reactants and products contain equal numbers of atoms in corresponding states of hybridization, have been successfully employed to obtain theoretical thermochemical values. Initially, the enthalpy of reaction was obtained by using Hess's Law and subsequently, the enthalpy of the goal molecule was also determined. The use of experimental values, in combination to those obtained from computational calculations has demonstrated to minimize the errors regarding the real value [106]. Thus, an isodesmic reaction that contains a large number of well known and consolidated experimental parameters is the best choice to obtain the thermochemical values.

Semi-empirical methods (AM1 and PM3) have been used to estimate the proton affinity of some organic compounds, such as amines and hydrocarbons [107]. The results successfully rationalized the formation of the ions that were observed in the ESI-MS spectrum [79,80]. However, with the advances in software and hardware, the use of more accurate computational methods has become viable [93,98-100]. Semi-empirical methods (PM5) have been used to calculate the stability of complexes of organic compounds with metal cations [108] and the results obtained were shown to be in concordance with the experimental ESI results.

Hammerun and Solling applied G2 (MP2) and CBS-Q to calculate the proton affinity of imines. They demonstrated that the calculated values differ from the experimental results by 0.5 kcal mol^{-1} and that, in the case of larger molecules, the CBS-Q method was more satisfactory [109]. DiLabio and co-workers developed a series of procedures similar to G2 as an alternative to density functional theory (DFT) calculations. These procedures consist of B3LYP calculations with large basis set (i.e., 6-31+G(2d,2p)) in combination with geometry and vibrational frequency optimization by conventional *ab initio* methods [110,111].

Although the PA and BG values can be obtained by computational methods, they are not sufficient to explain the generation of other ions in the ESI process that are not formed by protonation or desprotonation. Thus, other thermochemical parameters are required to rationalize the formation of all of the ions observed in the ESI-MS spectrum. In some circumstances, a competition occurs between the protonated species ($[\text{M}+\text{H}]^+$) and the molecular ion (M^+) [112]. Recent studies reported the generation of doubly charged radical ions using ESI and associated their formation with analyte oxidation potential values [52,72,73]. These studies showed that electron-withdrawing

groups can destabilize the positive radical ions by inductive effects [48]. Thus, an analysis of the effects of substituents on the stability of the resulting species is also needed.

A knowledge of ionization energies (IE) and electron affinities (EA) can contribute to an understanding of the ionic mechanisms in the gas-phase. As an example, the application of these theoretical parameters contributed to the interpretation of the MS spectra of several quaterthiophene-bridged bis-porphyrins [113].

In the case of coordination with metal cations (Na^+ or K^+) or even with anions (Cl^-), the intensity of the signal due to these species can be related to the cone potential (eV), which transfers kinetic energy to the species in the gas-phase [114,115]. Recently, an affinity scale that could assist in the interpretation of ESI-MS spectra with cationized/anionized molecules was tested [116]. Some molecules exhibited only ions resulting from coordination with metal cations in their spectra [117]. The search for the best site for accommodating the cation can be performed by the analysis of the atomic nucleophilic indices, more precisely by analysis of the Fukui function [118-120].

Computational methods can be employed to calculate the stability of the fragments obtained by in-source dissociation or even by collision-induced dissociation (CID). The energetic characteristics of the ions produced by ESI are different from those of ions produced by EI. In the case of ESI, the ions are guided toward the analyzers through a high pressure region and therefore, can suffer alterations in their internal energy. Studies using computational chemical models, for instance CBS-Q has been successfully used to explain the fragmentation mechanism and to elucidate the most favored fragmentation pathway [121]. In this sense, the use of computational thermochemistry, in combination with experimental ESI-MS data can contribute to a better interpretation of the ions that are produced in the gas-phase (i.e., by protonation/oxidation) as well as aid in the spectral interpretation [122].

5. CONCLUSIONS

Electrospray ionization mass spectrometry (ESI-MS) has been shown to be a powerful technique for generating protonated molecules ($[\text{M}+\text{H}]^+$), deprotonated molecules ($[\text{M}-\text{H}]^-$) and cationized molecules (i.e., $[\text{M}+\text{Na}]^+$). In some circumstances, however, the formation of radical ions (molecular ions) by removal ($\text{M}^{\bullet+}$) or addition ($\text{M}^{\bullet-}$) of one or more electrons, *via* redox reactions in the ES source can compete with acid/base reactions and coordination with ionic species. The extent to which these processes occur depends on a combination of factors, such as proton affinity and redox potential, as well as experimental parameters (flow rate of the solution into the capillary and solvent). Under well-defined conditions (compounds with low redox potential and low proton affinity and a low flow rate in a apolar solvent), the ES source can behave as a controlled-current electrolytic (CCE) flow cell, thus resulting in the formation of radical ions. In this context, computational methods have been successfully used in combination with experimental data to estimate some of the thermochemical quantities of interest, such as proton affinities, gas-phase basicities, ionization energies and redox potentials. Indeed, the formation of

radical ions represents not only expands in the potential of ESI-MS for the analysis of a wide variety of organic compounds, but also represents an interesting field of interdisciplinary research for analytical, physical, organic and theoretical chemists.

REFERENCES

- [1] Crews, P.; Rodrigues, J.; Jaspars, M. *Organic Structure Analysis*. Oxford University Press, Oxford, **1998**; pp. 295.T.
- [2] Pivatto, M.; Crotti, A. E. M.; Lopes, N. P.; Castro-Gamboa, I.; de Rezende, A.; Viegas, C.; Young, M. C. M.; Furlan, M.; Bolzani, V. S. *J. Brazil. Chem. Soc.*, **2005**, *16*, 1431.
- [3] Crotti, A. E. M.; Lopes, J. L. C.; Lopes, N. P. *J. Mass Spectrom.*, **2005**, *40*, 1030.
- [4] Wang, Y.; Song, F.; Xu, Q.; Liu, Z.; Liu, S. *J. Mass Spectrom.*, **2003**, *38*, 962.
- [5] Lopes, N. P.; Gates, P. J.; Wilkins, J. P. G.; Staunton, J. *Analyt.*, **2002**, *127*, 1224.
- [6] Fonseca, T.; Lopes, N. P.; Gates, P. J.; Staunton, J. *J. Am. Soc. Mass Spectrom.*, **2004**, *15*, 325.
- [7] Reale, S.; Di Tullio, A.; Spreti, N.; De Angelis, F. *Mass Spectrom. Rev.*, **2004**, *23*, 87.
- [8] Ding, W.; Johnson, K. A.; Kutal, C.; Amster, J. *Anal. Chem.*, **2003**, *75*, 4624.
- [9] Silva Jr., L. F.; Lopes, N. P. *Tetrahedron Lett.*, **2005**, *46*, 6023.
- [10] Dos Santos, M. D.; Martins, P. R.; dos Santos, P. A.; Bortocan, R.; Yamamoto, Y.; Lopes, N. P. *Eur. J. Pharm. Sci.*, **2005**, *26*, 62.
- [11] Guaratini, T.; Gates, P. J.; Cardoso, K. H. M.; Campos, P. M. B. G. M.; Colepicolo, P.; Lopes, N. P. *Eur. J. Mass. Spectrom.*, **2006**, *12*, 71.
- [12] McEwen, C. N.; Simonsick, W. J. Jr.; Larsen, B. S.; Ute, K.; Hatada, K. *J. Am. Soc. Mass Spectrom.*, **1995**, *6*, 906.
- [13] Dongré, A. R.; Somogyi, Á.; Wysocki, V. H. *J. Mass Spectrom.*, **1996**, *31*, 339.
- [14] Harrison, A. G.; Young, A. B. *J. Mass Spectrom.*, **2005**, *40*, 1173.
- [15] Jurchen, J. C.; Garcia, D. E.; Williams, E. R. *J. Am. Soc. Mass Spectrom.*, **2003**, *14*, 1373.
- [16] Yu, Y. W.; Stumpf, C. L.; Kenttämaa, H. I. *Int. J. Mass Spectrom.*, **2000**, *195*, 609.
- [17] Fenaillé, F.; Guy, P. A.; Tabet, J. C. *J. Am. Soc. Mass Spectrom.*, **2003**, *14*, 215.
- [18] Hudgins, R. R.; Woenckhaus, J.; Jarrold, M. F. *Int. J. Mass Spectrom. Ion Proc.*, **1997**, *165/166*, 497.
- [19] Paizs, B.; Suhai, S. *Mass Spectrom. Rev.*, **2005**, *24*, 508.
- [20] Todd, J. F. *J. Int. J. Mass Spectrom. Ion Proc.*, **1995**, *142*, 209.
- [21] Vestal, M. L. *Chem. Rev.*, **2001**, *101*, 361.
- [22] Crotti, A. E. M.; Vessecchi, R.; Lopes, J. L. C.; Lopes, N. P. *Quim. Nova*, **2006**, *2*, 287.
- [23] Cech, N. B.; Enke, C. G. *Mass Spectrom. Rev.*, **2001**, *20*, 362.
- [24] Fenn, J. B.; *Angew. Chem. Int. Ed.*, **2003**, *42*, 3871.
- [25] Fenn, J. B.; *J. Am. Soc. Mass Spectrom.*, **1993**, *4*, 524.
- [26] Yamashita, M.; Fenn, J. B. *J. Phys. Chem.*, **1984**, *88*, 4451.
- [27] Ikonomou, M. G.; Blades, A. T.; Kebarle, P. *Anal. Chem.*, **1991**, *63*, 1989.
- [28] Taflin, D. C.; Ward, T. L.; Davis, E. J. *Langmuir*, **1989**, *5*, 376.
- [29] Rohner, T. C.; Lion, N.; Giral, H. H. *Phys Chem. Chem Phys.*, **2004**, *6*, 3056.
- [30] Cole, R. B.; Ed. *Electrospray Mass Spectrometry: Fundamentals, Instrumentation & Applications*. New York: John Wiley & Sons., **1997**; pp. 65-105.
- [31] Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science*, **1989**, *246*, 64.
- [32] Crotti, A. E. M.; Carollo, C. A.; Gobbo-Neto, L.; Santos, M. D.; Gates, P. J.; Lopes, N. P. LC-Hyphenated techniques: uses in the structural elucidation of low- and high-molecular weight compounds. In *Modern Biotechnology in Medicinal Chemistry and Industry*. Carlton, A. Ed Taft, **2006**. Research Signpost, Kerala.
- [33] Kebarle, P.; Peschke, M. *Anal. Chim. Acta*, **2000**, *406*, 11.
- [34] Dole, M.; Mack, L. L.; Hines, R. L.; Mobley, R. C.; Ferguson, L. D.; Alice, M. B. *J. Chem. Phys.*, **1968**, *49*, 2240.

- [35] Iribarne, J. V.; Thomson, B. A. *J. Chem. Phys.*, **1976**, *64*, 2287.
- [36] Van Berkel, G. J.; Zhou, F. *Anal. Chem.*, **1995**, *67*, 3958.
- [37] Van Berkel, G. J.; Zhou, F. *Anal. Chem.*, **1995**, *67*, 2916.
- [38] De La Mora, J. F.; Van Berkel, G. J.; Enke, C. G.; Cole, R. B.; Martinez-Sanchez, M.; Fenn, J. B. *J. Mass Spectrom.*, **2000**, *35*, 939.
- [39] Van Berkel, G. J. *J. Mass Spectrom.*, **2000**, *35*, 773.
- [40] Xu, X.; Nolan, S. P.; Cole, R. B. *Anal. Chem.*, **1994**, *66*, 119.
- [41] Smith, D. P. H. *IEEE Trans. Ind. Appl.*, **1986**, *IA-22*, 527.
- [42] Hayati, I.; Bailey, A. I.; Tadeos, T. F. *J. Colloid Interface Sci.*, **1987**, *117*, 222.
- [43] Juhasz, P.; Ikonoumou, M. G.; Blades, A. T.; Kerbale. In *Methods and Mechanisms for Production ions from Large molecules*; Ens, W. Ed. Plenum Press, New York, **1991**.
- [44] de La Mora, J. F.; Loscertales, I. G. *J. Fluid Mech.*, **1994**, *260*, 155.
- [45] de La Mora, J. F. *J. Fluid Mech.*, **1992**, *243*, 561.
- [46] Meot-Ner, M. *Int. J. Mass Spectrom.*, **1998**, *33*, 212-220.
- [47] Dupont, A.; Gisselbrecht, J. P.; Leize, E.; Wagner, L.; Vandorselaer, A. *Tetrahedron Lett.*, **1994**, *35*, 6083.
- [48] Bard, A. J.; Ledwith, A.; Shine, H. J. In *Advances in Physical Organic Chemistry*; Gold, V.; Bethell, D. Eds.; Academic Press: New York, **1976**; Vol. 13, pp. 155-278.
- [49] Jayaweera, P.; Blades, A. T.; Ikonoumou, M. G.; Kebarle, P. *J. Am. Chem. Soc.*, **1990**, *112*, 2452.
- [50] Van Berkel, G. J.; McLuckey, S. A.; Glish, G. *Anal. Chem.*, **1992**, *64*, 1586.
- [51] Kertesz, V.; Van Berkel, G. J. *J. Mass Spectrom.*, **2001**, *36*, 204.
- [52] Van Berkel, G. J.; Giles, G. E.; Bullock, J. S. *Anal. Chem.*, **1999**, *71*, 5288.
- [53] Van Berkel, M. H.; Zhou, F. M.; Aronson, J. T. *Int. J. Mass Spectrom.*, **1997**, *162*, 55.
- [54] Amad, M. H.; Cech, N. B.; Jackson, G. S.; Enke, C. G. *J. Mass Spectrom.*, **2000**, *35*, 784.
- [55] Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science*, **1989**, *246*, 4926.
- [56] Shahat, A. A.; Cuyckens, F.; Wang, W.; Abdel-Shafeek, K. A.; Husseiny, H. A.; Apers, S.; Van Miert, S.; Pieters, L.; Vlietinck, A. J.; Claeys, M. *Rapid Comm. Mass Spectrom.*, **2005**, *19*, 2172.
- [57] Borsella, E.; Di Filippo, P.; Riccardi, C. *Ann. Chim.*, **2004**, *94*, 694.
- [58] de Kok, T. M.; Hogervorst, J. G.; Briede, J. J.; van Herwijnen, M. H.; Maas, L. M.; Moonen, E. J.; Driece, H. A.; Kleijnans, J. C. *Environ. Mol. Mutagen*, **2005**, *46*, 71.
- [59] Landis-Piowar, K. R.; Chen, D.; Cui, Q. C.; Minic, V.; Becker, F. F.; Banik, B. K.; Dou, Q. P. *Int. J. Mol. Med.*, **2006**, *17*, 931.
- [60] Marcoux, L. S.; Lomax, A.; Bard, A. J. *J. Amer. Chem. Soc.*, **1970**, *92*, 243.
- [61] Laali, K. K.; Hupertz, S.; Temu, A. G.; Galembeck, S. E. *Org. Biomol. Chem.*, **2005**, *3*, 2319.
- [62] Edge, R.; McGarvey, D. J.; Truscott, T. G. *J. Photochem. Photobiol. B Biol.*, **1997**, *41*, 189.
- [63] Pinto, E.; Catalani, L. H.; Lopes, N. P.; Di Mascio, P.; Colepicolo, P. *Biochem. Biophys. Res. Commun.*, **2000**, *268*, 496.
- [64] Rock, C. L. *Pharmacol. Ther.*, **1997**, *75*, 185.
- [65] Havaux, M. *Trends Plant Sci.*, **1996**, *3*, 147.
- [66] Koyama, Y. *J. Photochem. Photobiol. B Biol.*, **1991**, *9*, 265.
- [67] Britton, G. *FASEB J.*, **1995**, *9*, 1551.
- [68] Tian, L.; DellaPenna, D. *Arch. Biochem. Biophys.*, **2004**, *430*, 22.
- [69] Polivka, T.; Soundström, V. *Chem. Rev.*, **2004**, *104*, 2021.
- [70] (a) Van Breemen, R. B. *Anal. Chem.*, **1995**, *67*, 2004; (b) Ohashi, Y.; Itoh, Y. *Curr. Org. Chem.*, **2003**, *7*, 1605.
- [71] Guaratini, T.; Vessecchi, R. L.; Lavarda, F.; Maia-Campos, P.; Naal, Z.; Gates, P. J.; Lopes, N. P. *Analyst*, **2004**, *129*, 1223.
- [72] Guaratini, T.; Vessecchi, R.; Pinto, E.; Colepicolo, P.; Lopes, N. P. *J. Mass Spectrom.*, **2005**, *40*, 963.
- [73] Van Berkel, G. J.; Zhou, F. M. *J. Am. Soc. Mass Spectrom.*, **1996**, *7*, 157.
- [74] Kerbale, P.; Tang, L. *Anal. Chem.*, **1997**, *69*, 130.
- [75] Kealy, T. J.; Pauson, P. L. *Nature*, **1951**, *168*, 1039.
- [76] Janiak, C.; Schumann, H. *Adv. Organomet. Chem.*, **1991**, *33*, 291.
- [77] Stevens, A. E.; Beauchamp, J. L. *J. Am. Chem. Soc.*, **1981**, *103*, 190.
- [78] Friedman, L.; Irsa, I. P.; Wilkinson, G. J. *J. Am. Chem. Soc.*, **1955**, *77*, 3689.
- [79] Bejun, G. M.; Compton, R. N. *J. Chem. Phys.*, **1973**, *58*, 2271.
- [80] Van Berkel, G. J.; McLuckey, S. A.; Glish, G. L. *Anal. Chem.*, **1992**, *63*, 1098.
- [81] Van Berkel, G. J. *Anal. Chem.*, **1995**, *67*, 2916.
- [82] Fuhrhop, J. H.; Mauzerall, P. *J. Am. Chem. Soc.*, **1969**, *91*, 4174.
- [83] Monks, T. J.; Hanzlik, R. P.; Cohen, G. M.; Ross, D.; Graham, D. G. *Toxicol. Appl. Pharmacol.*, **1992**, *112*, 2.
- [84] Abreu, F. C.; Ferraz, P. A.; Goulart, M. O. F. *J. Braz. Chem. Soc.*, **2002**, *13*, 19.
- [85] Vessecchi, R. *Estudo do potencial redox e fragmentação em ES-EM/EM de 1,4-naftoquinonas* (Dissertation in Portuguese). Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, **2005**.
- [86] Kertesz, V.; Van Berkel, G. J. *J. Sol. State Electrochem.*, **2005**, *9*, 390.
- [87] Hauffer, R. E.; Conceição, J.; Chibante, L. P. F.; Chai, Y.; Byrne, N. E.; Flanagan, S.; Haley, M. M.; O'Brien, S. C.; Pan, C.; Xiao, Z.; Billups, W. E.; Ciufolini, M. A.; Hauge, R. H.; Margrave, J. L.; Wilson, L. J.; Curl, R. F.; Smalley, R. E. *J. Phys. Chem.*, **1990**, *94*, 8634.
- [88] Dubois, D.; Moninot, G.; Kutner, W.; Jones, M. T.; Kadish, K. M. *J. Phys. Chem.*, **1992**, *96*, 7137.
- [89] Hettich, R.; Jin, C.; Compton, R. N. *Int. J. Mass Spectrom.*, **1994**, *137*, 263.
- [90] Zhou, F.; Van Berkel, G. J.; Donovan B. T. *J. Am. Chem. Soc.*, **1994**, *116*, 5485.
- [91] Meot-Ner, M. *Int. J. Mass Spectrom.*, **2003**, *227*, 525.
- [92] Gal, J. F.; Maria, P. C.; Raczyńska, E. D. *J. Mass Spectrom.*, **2000**, *36*, 699.
- [93] Bell, R. P. *The Proton in Chemistry*. Cornell University Press: Ithaca, NY, **1959**.
- [94] (a) Cooks, R. G.; Wong, P. S. H. *Acc. Chem. Res.*, **1998**, *31*, 379; (b) Augusti, R.; Augustu, D. V.; Chen, H.; Cooks, R. G. *Eur. J. Mass Spectrom.*, **2004**, *10*, 847.
- [95] Wu, L. M.; Tao, W. A.; Cooks, R. J. *J. Mass Spectrom.*, **2003**, *38*, 386.
- [96] Armentrout, P. B. *J. Mass Spectrom.*, **1999**, *34*, 74.
- [97] Curtiss, L. A.; Raghavachari, K.; Redfern, P. C.; Kedziora, G. S.; Pople, J. A. *J. Phys. Chem. A*, **2001**, *105*, 227.
- [98] Ventura, O. N.; Kieninger, M. *Pure Appl. Chem.*, **1998**, *70*, 2301.
- [99] Bouchoux, G.; Drancourt, D.; Leblanc, d.; Yanez, M.; Mo, O. *New J. Chem.*, **1995**, 1243.
- [100] Maksic, Z. B.; Vianello, R. *J. Phys. Chem. A*, **2002**, *106*, 419.
- [101] Deakne, C. A. *Int. J. Mass Spectrom.*, **2003**, *227*, 601.
- [102] Calvin, E.; Gold, V. Eds. *Proton-Transfer Reactions*. Chapman and Hall: London, **1975**.
- [103] Stewart, R. *The Proton: Application to Organic Chemistry*. Academic Press: Orlando, FL, **1985**.
- [104] Fattahi, A.; Kass, S. R.; Liebman, J. F.; Matos, M. A. R.; Miranda, M. S.; Morais, V. M. F. *J. Am. Chem. Soc.*, **2005**, *127*, 6116.
- [105] Ventura, O.; Segovia, M. *Chem. Phys. Lett.*, **2005**, *403*, 378.
- [106] Denis, P. A.; Ventura, O. N. *Int. J. Quantum Chem.*, **2000**, *80*, 439.
- [107] Dewar, M. J. S.; Dieter, K. M. *J. Am. Chem. Soc.*, **1986**, *108*, 8075.
- [108] Lyapchenko, N.; Eitner, K.; Schroeder, G.; Brzezinski, B. *J. Mol. Struct.*, **2004**, *690*, 45.
- [109] Hammerun, S.; Solling, T. I. *J. Am. Chem. Soc.*, **1999**, *121*, 6002.
- [110] Di Labio, G. A.; Pratt, D. A.; Wright, J. S. *Chem. Phys. Lett.*, **1999**, *311*, 215.
- [111] Foresman, J. B.; Frisch, J. B. *Exploring Chemistry with Electronic Structure Methods*. 2th Edition, Gaussian Inc., Pittsburg, **1996**; pp. 301.
- [112] Dezhong, L.; Gao, Y.; Kispert, L. W. *J. Electroanal. Chem.*, **2000**, *48*, 140.
- [113] Rondeau, D.; Rogalewicz, F.; Ohanessian, G.; Levillain, E.; Odobel, F.; Richomme, P. *J. Mass Spectrom.*, **2005**, *40*, 628.
- [114] Asthon, D. S.; Beddell, C. R.; Cooper, D. J.; Grenn, B. N.; Oliver, R. W. A. *Org. Mass Spectrom.*, **1993**, *28*, 721.
- [115] Gabeliua, V.; De Pauw, E. *Mass Spectrom. Rev.*, **2005**, *24*, 566.
- [116] Petrie, S. *J. Phys. Chem. A*, **2001**, *105*, 9931.

- [117] Lopes, N. P.; Stark, C. B. W.; Gates, P. J. *Analyst*, **2002**, *127*, 503.
[118] Galembeck, S. E.; Camaroni, G. F. *Quim. Nova*, **2003**, *26*, 957.
[119] Contreras, R. R.; Fuentealba, P.; Galvan, M.; Perez, P. *Chem. Phys. Lett.*, **1999**, *304*, 405.
[120] Melin, J.; Aparicio, F.; Subramanian, V.; Galvan, M.; Chattaraj, P. K. *J. Phys. Chem. A*, **2004**, *108*, 2487.
[121] Crotti, A. E. M.; Fonseca, T.; Hong, H.; Staunton, J.; Galembeck, S. E.; Lopes, N. P.; Gates, P. J. *Int. J. Mass Spectrom.*, **2004**, *232*, 271.
[122] Vessecchi, R.; Nascimento, P. G. B. D.; Lopes, J. N. C.; Lopes, N. P. J. *J. Mass Spectrom.*, **2006**, *14*, 1219.

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