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Redox transformations in desorption electrospray ionization

Mario Benassi[1], Chunping Wu, Marcela Nefliu, Demian R. Ifa, Michael Volný[2], R. Graham Cooks*

Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, IN 47907, USA

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Dedicated to Professor Zdeněk Herman on the occasion of his 75th birthday and in appreciation of his deep and diverse contributions to science.

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ABSTRACT

Redox changes occur in some circumstances when organic compounds are analyzed by desorption electrospray ionization mass spectrometry (DESI-MS). However, these processes are limited in scope and the data presented here suggest that there are only limited analogies between the redox behavior in DESI and the well-known solution-phase electrochemical processes in standard electrospray ionization (ESI). Positive and negative ion modes were both investigated and there is a striking asymmetry between the incidence of oxidation and of reduction. Although in negative ion mode DESI experiments, some aromatic compounds were ionized as odd-electron anion radicals, examples of full reduction were not found. By contrast, oxidation in the form of oxygen atom addition (or multiple oxygen atom additions) was observed for several different analytes. These oxidation reactions point to chemically rather than electrochemically controlled processes. Data is presented which suggests that oxidation is predominantly caused by reaction with discharge-created gas-phase radicals. The fact that common reducing agents and known antioxidants such as ascorbic acid are not modified, while a saturated organic acid like stearic acid is oxidized in DESI, indicates that the usual electrochemical redox reactions are not significant but that redox chemistry can be induced under special experimental conditions.

1. Introduction

Electrospray mass spectrometry (ESI-MS) is one of the most widely used analytical techniques, allowing the analysis of solutions and impacting diverse areas of science and industry. Desorption electrospray ionization (DESI) [1] is an ambient surface analysis method that shares some of the properties of the electrospray technique. Solvent nebulization to create the primary projectile droplets in DESI uses standard electrosonic spray ionization (ESSI) [2] sources and the progeny droplets generated in the course of surface collisions go on to yield gas-phase ions by processes similar to those involved in the electrospray ionization process. In DESI, aqueous droplets with diameters less than 10 μm impact on a sample surface at velocities typically in excess of 100 m/s. Droplet dynamics measurements and simulations indicate that a droplet-pickup mechanism involving analyte extraction into a thin film of solution on the surface likely operates under most circumstances [3]. The dissolved analyte is desorbed in a momentum transfer event (a "splash") with newly arrived primary droplets. The released droplets take the form of charged sub-micrometer droplets that are converted into free gas-phase ions by standard ESI desolvation mechanisms.

The occurrence of electrochemical processes in ESI might be considered "too obvious to mention" [4], but the fact that electrochemical processes in ESI do not hinder its practical usefulness may be attributed to a fortuitous choice of electrode materials, configuration of source operating parameters, and the original selection of analytes that are not susceptible to electrochemical modification [5]. The underlying processes in ESI, which include the electrochemical processes inherent in the operation of an electrospray, are now much better understood than at the time of the first coupling of ESI with a mass spectrometer. The general electrospray mechanism [6–9,12,20] as well as electrochemical redox processes in ESI [5,10,11,15,16,18,19] have been investigated and the origin of radical ions explained by the electrochemical nature of the electrospray [17]. Other studies focused on current measurements and charge buildup [13,4] during ESI process. It is now known that the interfacial potential [21] of the emitter electrode is not fixed during ESI operation (unlike the constant high voltage on the spray emitter) and that it depends on several parameters (related to solution composition, flow rate and emitter electrode properties). It is highest at the spray tip (estimated from simulations as <2.5 V) [21]

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* Corresponding author. Tel.: +1 765 494 5262; fax: +1 765 494 9421.
E-mail address: cooks@purdue.edu (R.G. Cooks).

[1] On leave from ThoMSon Mass Spectrometry Laboratory, State University of Campinas, Campinas, SP 13085-970, Brazil.

[2] Current address: Laboratory of Molecular Structure Characterization, Academy of Sciences of the Czech Republic, Videnska 1083, Prague 4, 14220 Czech Republic.

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and decreases upstream into the capillary [14,21,22]. Thus, other competing processes (solvent system reactions or capillary surface redox processes) can successfully function as redox buffers and minimize the redox changes of the analyte of interest by reducing the amount of Faradaic current available for further electrochemical reaction. There are also other limitations: heterogeneous electron transfer chemistry cannot occur unless the analyte is transported to the surface of the electrode and then back again (to be detected in the spectrum the new species must also be stable enough to reach the mass analyzer). Analyte can also react with electrochemically generated species that originate from the solvent system and dissolved gases, but the time for homogenous reactions with dilute analytes in flow systems is limited. All these factors contribute to the fact that electrochemical transformations of the analyte in ESI are not very common and are of only relatively minor significance during routine analytical usage, especially for organic and biological compounds. However, the exceptions to this claim are of a great interest and there are now known to be several classes of analytes that undergo electrochemical reactions in the course of electrospray ionization mass spectrometry [5]. Still other analytes can have their mass spectra significantly impacted by electrochemical changes in the mobile phase composition (e.g., changes in pH) [23].

It has been demonstrated that analytes in DESI can undergo unexpected oxidation [24] or reduction, in the latter case being ionized in to form an odd-electron anion radical [25], a process that in electrospray is traditionally attributed to the electrochemical nature of ESI [6,17,20,26]. In a previous report [27], we investigated the influence of the surface on the electrical processes in DESI by measuring total current (sum of all charged species created by electrospray emitter and reflected from the DESI surface) between the sample surface and an electrode placed at the instrument inlet. We found that during a DESI experiment the total current initially rises sharply, followed by an exponential decrease to a steady state. The current drops to negative values when the high voltage on the spray emitter is switched off, strongly suggesting that the direction of current flow in the equivalent DESI circuit is reversed. This demonstrates that the DESI source behaves as a DC capacitor and that the addition of a surface between the sprayer and the counter electrode in DESI introduces a new electrically active element into the chemical analysis system. We also detected partial oxidation of some analytes during our earlier experiments. Because the ambient laboratory environment is chiefly oxidative, in this report we enlarged our focus by studying reduction in negative ion DESI mode and tried to eliminate confusion from other possible means of oxidation by using labeled water and degassed solvents. In order to determine the influence of the surface on the analyte, DESI is here compared with its parent ESSI technique [2] rather than with standard ESI.

2. Experimental

2.1. Materials and reagents

All solutions were prepared using water deionized at 18.2 MΩ cm prepared by a Milli-Q purification system (Millipore) and HPLC grade organic solvents purchased from Mallinckrodt. L-Ascorbic acid was supplied by J.T. Baker. Phenol was acquired from Mallinckrodt and hydroquinone from Fisher Scientific. The compound 16:0–18:1 PC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) was purchased from Avanti Polar Lipids, Inc. Oxygen labeled $H_2{}^{18}O$ and $H_2{}^{18}O_2$ were obtained from Sigma–Aldrich. All other chemicals were purchased from Sigma–Aldrich. Porous polytetrafluoroethylene (PTFE) sheet, of 1/16 in. thickness, was purchased from Small Parts Inc. (Miami Lakes, FL) and used as the substrate in DESI.

Table 1
Redox behavior in negative ion mode DESI-MS.

(1) Compounds that do not undergo redox change	(2) Compounds that do undergo redox change
(1A) Oxidized forms	(2A) Ionize to give anion radicals
Retinoic acid (all trans)	Benzoquinone
Nicotinamide adenine dinucleotide	Benzoquinone-d_4
	Trinitrotoluene [25]
(1B) Reduced forms	(2B) Undergo oxidation by oxygen attachment
L-Ascorbic acid	Stearic acid
Hydroquinone	
Catechol	
Phenol	
Cannabidiol	

2.2. DESI-MS

The setup used for DESI current measurements utilized (see also supplementary data) a Keithley 6485 pico-ammeter and was identical to that described in our previous report [27]. By current, we mean the spray current that hits the Cu plate after the secondary droplets leave the DESI surface (supplementary data). All mass spectra were acquired using a commercial Thermo Finnigan LTQ (San Jose, CA) linear ion trap mass spectrometer. Our ESSI source has been described in the literature [2] and the DESI source coupled to the LTQ was either a standard commercial DESI source (Prosolia) or its prototype built in our lab. A flow rate of 3 μL/min, capillary temperature of 150 °C and a spray impact angle of 55° were used. The nebulizing gas (N_2) pressure was 150 psi.

3. Results

3.1. Negative mode DESI-MS

The reduction of analyte in negative ion mode ESI has been described for several types of compounds (fullerenes, organometallics, aromatics and nitro compounds) [5]. There is an asymmetry between the positive and negative electrospray modes with oxidation in the positive mode being much more common, although reduction is still observed. By contrast, our results indicate that reduction in DESI is extremely rare. Table 1 shows a list of compounds that did not undergo any redox change in negative mode DESI. Interestingly, it includes nicotinamide adenine dinucleotide (NAD^+) which is an oxidized form of an important biological charge carrier and an oxidizing agent. The oxidized form did not undergo reduction when analyzed by DESI in the negative ion mode; the analyte was simply detected as the $[M–H]^-$ ion as is typical for negative ion mode ESI and DESI. This shows that the negative charge on the surface that develops there during a DESI experiment has no redox consequence, even for an electrochemically sensitive compound. As another test, we investigated the DESI behavior of 1,4-benzoquinone. It is well known that benzoquinone and hydroquinone undergo electrochemical reactions during electrospray [5]. Based on the ESSI behavior (Fig. 1A) in the negative ion mode it was expected that 1,4-benzoquinone would be reduced in DESI to form 1,4-hydroquinone and then be ionized to yield the $[M–H]^-$ even-electron ion of m/z 109 (Scheme 1). Instead, as can be seen in Fig. 1B, an odd-electron radical anion of benzoquinone was observed. This result was confirmed by performing the same experiment with labeled 1,4-benzoquinone-d_4 (Fig. 2). This difference in ESSI/DESI behavior is obviously associated with the presence of the extra (sample) surface in DESI although the reason is not entirely clear.

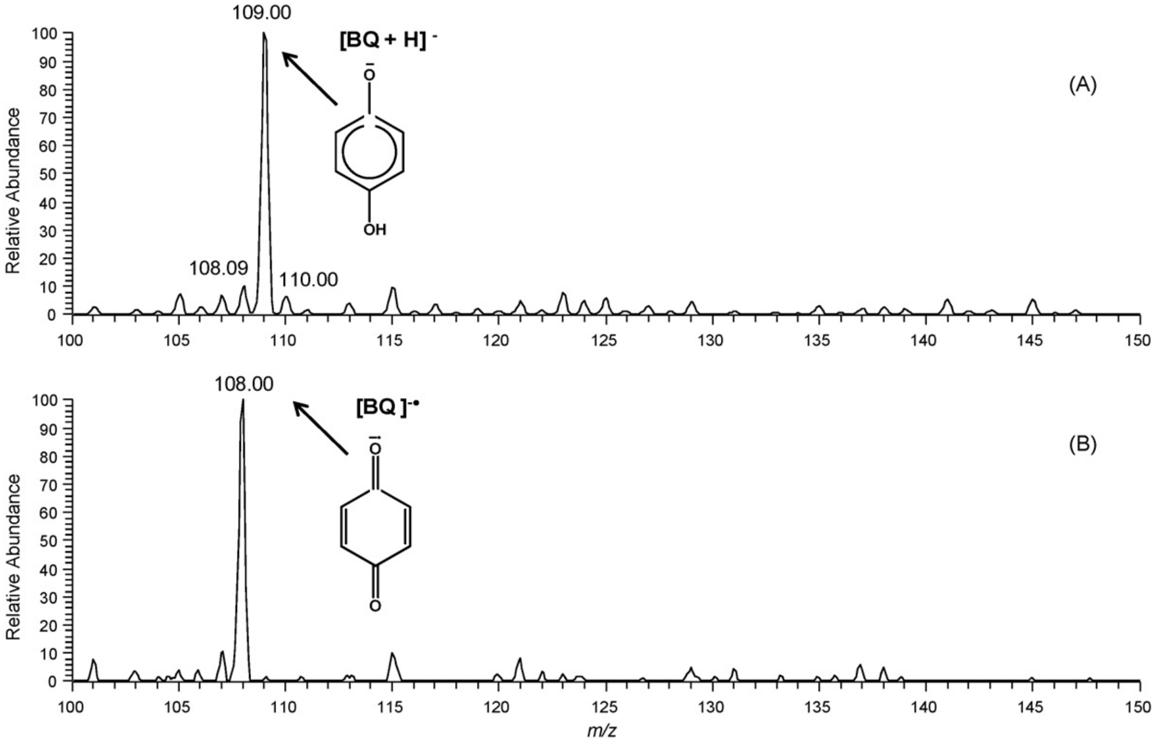


Fig. 1. Mass spectra of 1,4-benzoquinone (BQ) (108 Da) in the negative ion mode using ESSI (A) and DESI on a PTFE surface (B). For the ESSI experiments a 250 μg/mL solution of methanol/water (1:1, v/v) was directly infused at 5 μL/min and 5 kV spray voltage. DESI experiments were conducted using 10 μg sample spots on PTFE at 5 kV spray voltage, using methanol/water (1:1, v/v) as spray solution at 5 μL/min. The DESI spray was positioned 0.5–1 mm above the sample surface.

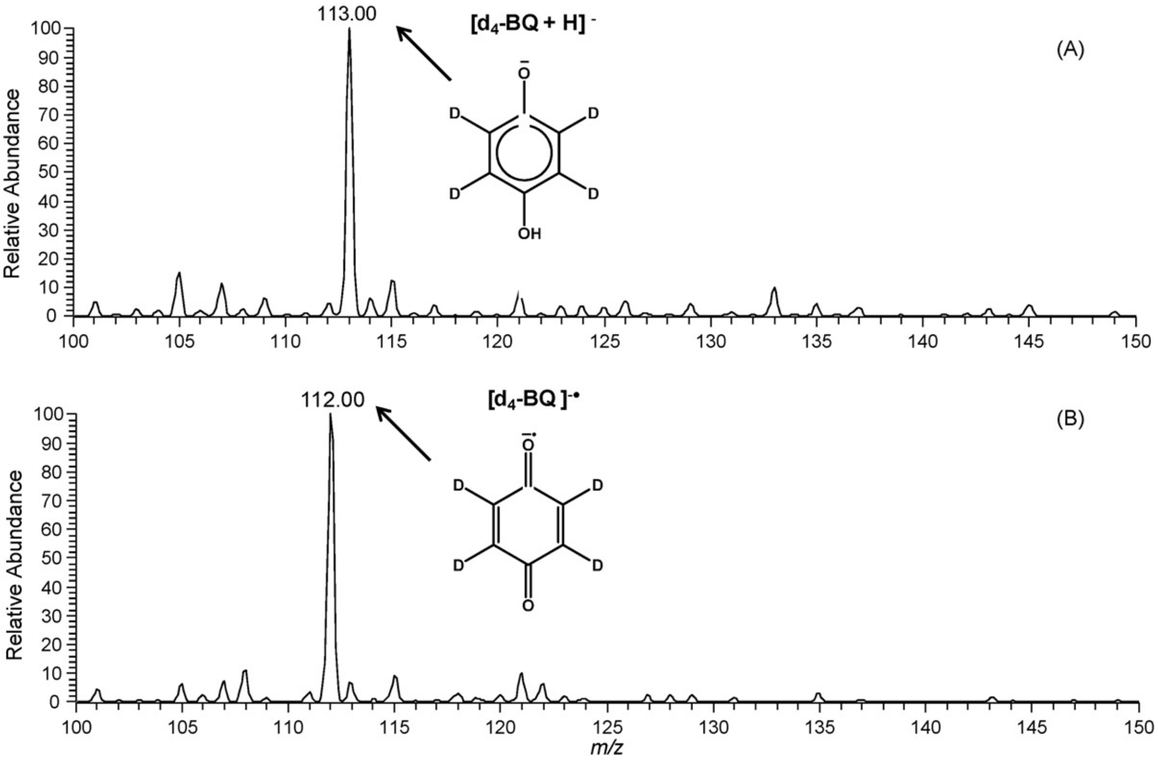
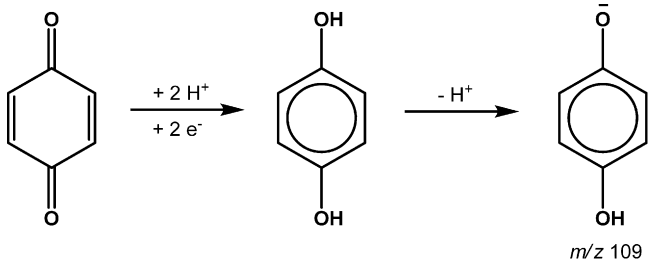


Fig. 2. Mass spectra of 1,4-benzoquinone-d$_4$ (d$_4$-BQ) (112 Da) in the negative ion mode using ESSI (A) and DESI on a PTFE surface (B). For the ESSI experiments a 250 μg/mL solution of methanol/water (1:1, v/v) was directly infused at 5 μL/min and 5 kV spray voltage. DESI experiments were conducted using 10 μg sample spots on PTFE at 5 kV spray voltage, using methanol/water (1:1, v/v) as spray solution at 5 μL/min. The DESI spray was positioned 0.5–1 mm above the sample surface.



Scheme 1. Reduction of benzoquinone in ESSI.

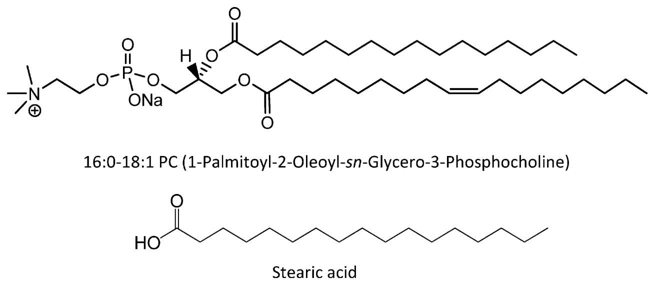
Similar behavior was previously described for the explosive TNT, which also forms an anion radical in negative ion mode DESI [25].

It is interesting to note that while retinoic acid (oxidized form of vitamin A) did not undergo any redox transformation in DESI, oxidation was observed in the negative ion mode for stearic acid (Scheme 2), a saturated acid that lacks any obvious functional group for oxidation. Fig. 3 shows the ESSI and the DESI mass spectra obtained in the negative ion mode. Negligible oxidation occurs in ESSI while in DESI up to five oxidation steps can be observed. This observation is discussed later but it clearly shows that a heterogeneous electron transfer reaction, or a reaction with electrochemically generated species, is not responsible for this particular oxidation reaction. If solution-phase redox reactions did occur, then in the negative ion mode both ionization methods would favor reduction of the analyte. It is also worth noting that DESI experiments performed on the reduced form of ascorbic acid in the negative ion mode did not result in any oxidation. The possible further reduction was not observed either. Thus ascorbic acid is simply ionized by proton abstraction to form the $[M{-}H]^-$ ion of *m*/*z* 175 without any redox change. The fact that common reducing agents and known antioxidants, such as ascorbic acid, remain unaffected while a saturated organic acid is oxidized indicates an unusual and chemically specific mechanism whereby the saturated alkyl chain in stearic acid is oxidized rather than a normal electrochemical process.

We also investigated charging of the surface during negative ion mode DESI experiments and the data together with a brief discussion are available as part I of the supplemental material.

3.2. Positive mode DESI-MS

The oxidation of analyte in the positive ion mode in DESI, leading mainly to the net addition of one or more oxygen atoms to the analyte molecules, was previously reported for several different organic compounds [24]. Oxidation was attributed to several factors including reaction with reactive oxygen species generated by the electrospray process and proof has been provided that methanol:water DESI spray mixture contains electrochemically generated peroxide anions. Here we present experiments in



Scheme 2. Structures of stearic acid and 16:0–18:1 PC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine).

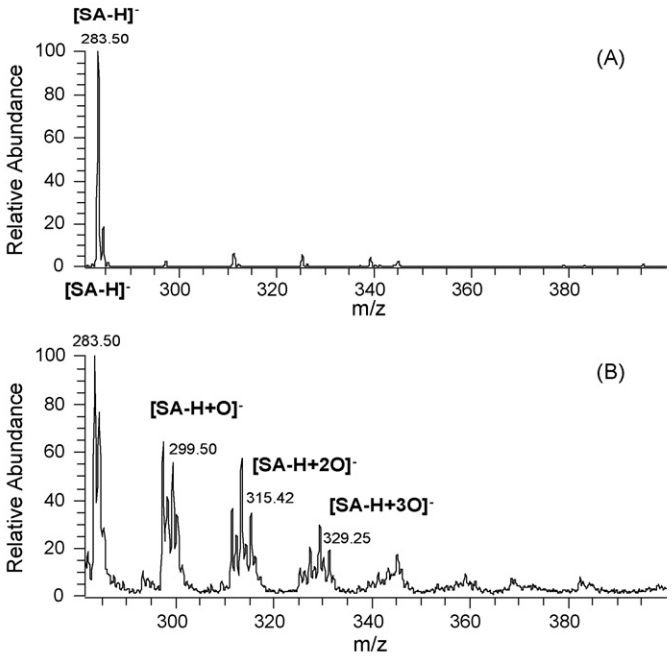


Fig. 3. Negative ion ESSI (A) and DESI (B) mass spectra of stearic acid (SA). For the ESSI experiment a 100 μg/mL solution in acetonitrile/water (3:1, v/v) was directly infused at 3 μL/min and 5 kV spray voltage. DESI experiments were conducted using 2 μg sample spots on PTFE at 5 kV spray voltage, using acetonitrile/water (3:1, v/v) as solvent spray at 3 μL/min. The DESI spray was positioned 0.5–1 mm above the sample surface.

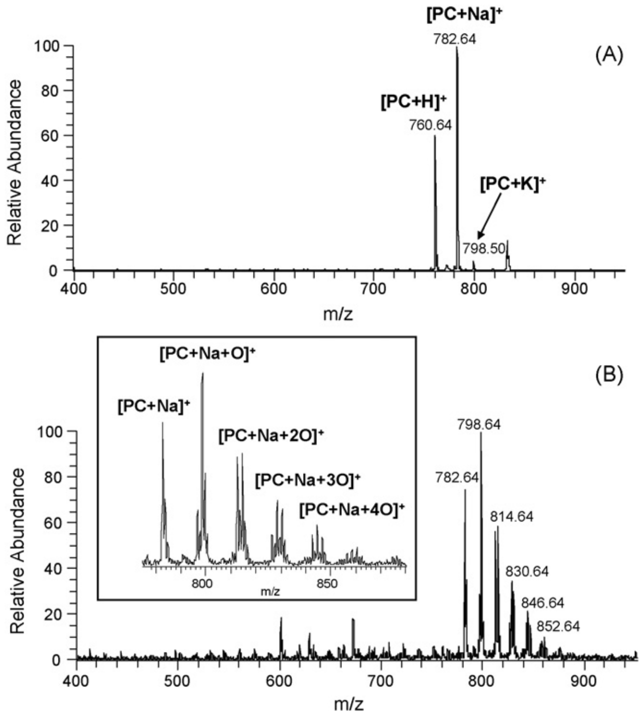


Fig. 4. Positive ion ESSI (A) and DESI (B) mass spectra of 16:0–18:1 PC. ESSI experiment was conducted using a 50 μg/mL PC solution in acetonitrile, at 5 kV spray voltage and 3 μL/min flow rate. For the DESI experiment, 2 μg material was deposited on PTFE and sprayed with degassed acetonitrile at 3 μL/min and 5 kV spray voltage. The inset shows the magnified *m*/*z* range 775–870.

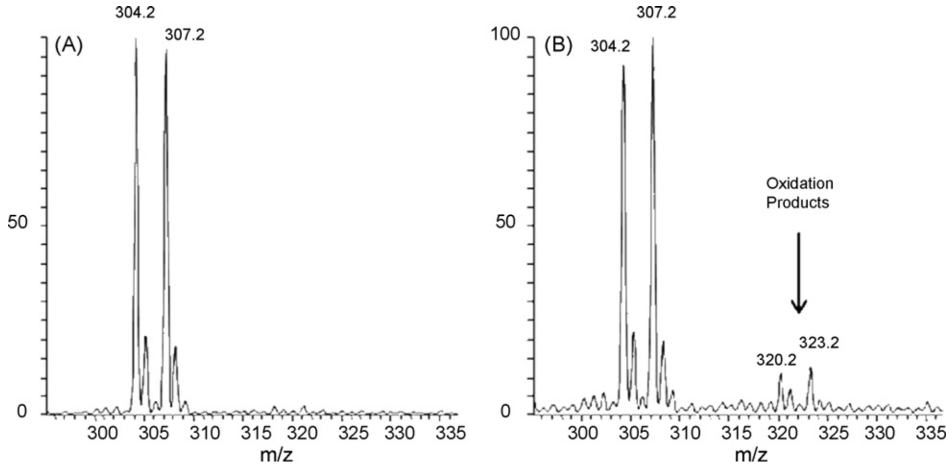


Fig. 5. DESI of cocaine using different spray voltages: 4 kV (A) and 8 kV (B). Signals of *m*/*z* 304.2 and 307.2 represent proton adducts of cocaine and deuterium labeled cocaine-d_3 internal standard. Signals of *m*/*z* 320.2 and 323.2 are the corresponding oxidation products (surface PTFE, spray mixture MeOH:H_2O (1:1)).

which we seek additional insights into analyte oxidation in DESI. Fig. 4A shows the positive ion ESSI mass spectrum of 16:0–18:1 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (abbreviated to PC, Scheme 2), recorded at 5 kV spray voltage. The signal of *m*/*z* 798 is the PC potassium adduct, as confirmed through tandem MS. Fig. 4B shows the positive ion DESI mass spectrum of the same compound obtained when 2 μg of material was deposited on a PTFE surface sprayed with degassed acetonitrile at 3 μL/min and 5 kV spray voltage. Up to six additions of units with mass 16 Da can be observed along with losses of 2 Da or even 4 Da. The observed multiple additions of 16 Da in the spectrum of a singly charged PC and stearic acid suggest that multiple oxygen atoms are added in the form of hydroxyl groups to the aliphatic chain. Ozonolysis is also possible, because PC contains a double bond in the position 18 of the aliphatic chain. The ozone electrospray ionization (OzESI-MS) of PC was investigated by the Blanksby group [28]. However, in their study, no addition of 16, 32 or 48 was observed when a standard ESI was coupled to a commercial ozone generator, which led them to a conclusion that stable ozonides are not created by a direct reaction between ozone and PC in electrospray.

We also investigated the effects of other experimental parameters including solvent composition, sprayer position in relation to the sample and the MS inlet, and the sprayer design. As previously reported [24], the spray voltage and the analyte concentration affect the extent of oxidation and our results agree with these observations. The effect of water and hydrogen peroxide were tested by adding $H_2{}^{18}O$ and $H_2{}^{18}O_2$ to degassed acetonitrile. Although the products of oxidation were still detected, the increase in mass corresponded to additions of ^{16}O and not ^{18}O, suggesting that the oxidation reactions do not involve the water or the hydrogen peroxide formed from the water in the solvent. The distance between the sprayer and the sample surface proved to have an important effect in controlling oxidation. The spectrum in Fig. 4B was acquired at a distance of about 0.5 mm above the sample (as close as practically possible). At about 2 mm the extent of oxidation decreases and DESI spectra are very similar to those obtained by ESSI. The distance between the sprayer tip and the MS inlet appears to have a smaller effect and experiments conducted at 1–2 mm provided optimum redox signals. The fact that geometric parameters play such a critical role indicates that oxidizing species present in the solvent spray that remains unchanged over the entire experiment may not be the key reaction partners. On the other hand, stronger electric discharges can be induced at smaller distances between the sprayer and the sample surface, accounting for the distance effect.

The facts reported here (the negligible effect of water and hydrogen peroxide contained in the solvent spray, the effect of spray-to-surface distance, the observation of oxidation products in the negative ion mode) suggest that the electrochemistry alone (and by that we refer to the effect of redox species generated electrochemically in the solvent spray) cannot explain the multiple oxidations observed in DESI. After ruling out direct heterogeneous electron transfer, reaction with oxygen reactive species in solution, reaction with hydrogen peroxide and reaction with ozone, gas-phase reaction with discharge-generated radicals is suggested by a process of elimination to account for these results. It has been reported that hydroxyl radical can induce oxidation of peptides and proteins in electrospray coupled with corona discharge [29]. It is also known from studies of atmospheric chemistry [30], that ion radicals can react with molecular oxygen, a process which results in direct oxygen attachment. Such reactions can however only explain even-numbered and not odd-numbered oxygen adducts.

Another example of oxidation occurred for a mixture of cocaine/cocaine-d_3 in DESI (Fig. 5). The data clearly show the presence of oxidized species of *m*/*z* 320 and 323, respectively, under higher voltage operation. But it is important to point out that cocaine oxidation only appears at very high spray potential (8 kV) far from the normal voltage used in analytical DESI. The supplemental material includes data that show that the oxidation of cocaine also depends on the nature of the surface material and that it increases after addition of 5 mM ammonium acetate to the spray mixture (Figures S2 and S3). Similar results were obtained for heroin and morphine (Figure S4). Unlike the case of stearic acid (Fig. 3) and PC (Fig. 4) both of which oxidize relatively easily, DESI oxidation of selected alkaloids required relatively harsh conditions (voltage, high electrolyte concentration). The practical aspect of this finding is that it should not be difficult for an analytical chemist to avoid oxidation of these compounds in DESI by selecting more gentle experimental conditions. It is not clear, however, if the basic heterocyclic compounds, such as isoquinoline and tropane group alkaloids investigated here, oxidize by the same mechanism as the less polar fatty acids or lipids that generally undergo oxidation much more readily in our experiments.

4. Conclusion

We have investigated DESI behavior of a group of structurally diverse compounds to gain a better understanding of the occurrence of redox processes in DESI and their underlying mechanisms. It is known from traditional electrospray experiments that oxidation in the positive ion mode is more common than reduction in the negative mode. This asymmetry is even more dominant in DESI. We only found a limited number of reduction processes in DESI while oxidation can be observed relatively easily for different classes of compounds. Our study also revealed differences between redox transformation of analyte in DESI and ESI, although we used ESSI instead of conventional ESI to more closely match the technique with DESI. We discovered that the occurrence of oxidation in DESI does not correlate with electrochemical properties but it does show chemical specificity. For example, it occurs for the saturated carboxylic acid, stearic acid, but not for easily oxidized (in solution) hydroquinone. We conclude that redox transformations in DESI are complex processes and that standard solution-phase electrochemical processes have only limited influence. The most intense redox process in DESI is addition of oxygen atom (or multiple oxygen atoms) to the analyte molecule. Although more investigation is needed, we suspect that this is a plasma process which is predominantly caused by reaction with discharge-created radicals in the gas phase.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2008.10.012.

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