

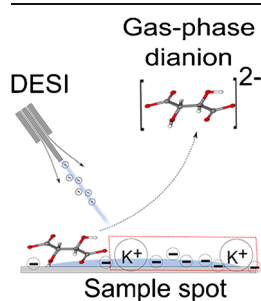
High Production of Small Organic Dicarboxylate Dianions by DESI and ESI

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Abstract. A significant production of gas-phase dicarboxylate dianions has been observed in standard ESI and DESI during the analysis of small organic dicarboxylic acids under moderate or highly alkaline conditions. In ESI, this can be attributed to an excess of hydroxyl ions (OH^-) which favor the formation of a high amount of dianions in solution, contemporarily trapping the potential counter ions during the ESI process. The results obtained in DESI highlight the role of the surface in trapping the counterions during desorption process and determining the ultimate nature of the observed gas-phase ions.

Keywords: Dicarboxylates, Gas-phase dianions, ESI, DESI, Mass spectrometry

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Introduction

Small organic dicarboxylic acids play an important role in many biological systems. In the case of plants, they are key intermediates in carbon metabolism and may be present in high concentrations—often stored as K^+ salts [1]—with important implications for the production of beverages like juices or wine.

Since the $\text{pK}_{\text{a}2}$ values in water of dicarboxylic acids (H_2DCA), such as succinic, glutaric, adipic, malic, and tartaric acids, are lower than six [2, 3], in alkaline aqueous solutions they are expected to be present mostly as dicarboxylate dianions (DCA^{2-}), with relatively minor amounts of the monoprotonated HDCA^- species. Despite this, small DCA^{2-} species are rarely (and barely) observed in the gas phase and it is speculated that this is due to the absence of the interactions with surrounding media (i.e., counterions or solvation). This type of interaction is always required to stabilize the charges [4–6]. From a general point of view, DCA^{2-} species can disappear through three main mechanisms: (1) protonation during solvent evaporation leading to HDCA^- ; (2) one electron

loss leading, through an intermediate radical anion, to a decarboxylated singly charged radical anion [7, 8]; or (3) dissociation into singly-charged negative ions.

For a given carbon backbone, the stability of DCA^{2-} in the gas phase can be increased by introducing additional functional groups, which allow charge delocalization and/or favor intramolecular hydrogen bonding, as in the case of tartaric acid [7]. This multifaceted behavior, coupled with a relatively simple structure, has made H_2DCA an ideal system for studying fundamental molecular phenomena like Coulomb repulsion [5] and solute-solvent interactions [6, 9]. From an experimental point of view, ESI should be the favored technique for producing gas phase DCA^{2-} , but it has always been quite difficult to produce these ionic species in significant amounts [10], at least for H_2DCA with low molecular weight. In particular, a recent study by Tonner et al. [7] shows that tartaric dianions (TA^{2-}) can be produced by ESI, although not very efficiently, only under restricted experimental conditions, whereby their spontaneous thermodynamic decomposition via mechanism (b) is somewhat hindered. These calculations indeed indicate that TA^{2-} is metastable with respect to dissociation into the decarboxylated radical anion + $\text{CO}_2 + \text{e}^-$.

Since it is widely accepted that desorption electrospray ionization (DESI) follows ESI-like dynamics [11–13], the production of TA^{2-} species in DESI-MS spectra was expected to

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be also highly inefficient. However, during DESI-MS imaging of H₂TA in grape tissues (Figure 1), we observed a relatively strong signal at *m/z* 74.00 attributable to the tartrate dianion. This assignment was confirmed by high resolution measurements and isotope cluster analysis, which unambiguously assigned to this ion the C₄H₄O₆²⁻ molecular formula. Therefore, TA²⁻ species could be produced in good yields under standard DESI-MS conditions. This outcome seemed to suggest the presence of significant and subtle differences between DESI and ESI for dianion production.

In order to investigate the DCA²⁻ formation mechanism, we designed a series of DESI and ESI experiments on H₂DCA species of different carbon backbone lengths and with different numbers of hydroxyl groups. In particular, we analyzed: (a) five H₂DCA acids of general structural formula HOOC-(CH₂)_{*n*}-COOH: succinic (*n* = 2, H₂SA), glutaric (*n* = 3, H₂GA), adipic (*n* = 4, H₂AA), pimelic (*n* = 5, H₂PA), and suberic acids (*n* = 6, H₂SuA); (b) three hydroxylated dicarboxylic acids: tartronic (HOOC-CHOH-COOH, H₂TrA), malic (HOOC-CH₂-CHOH-COOH, H₂MA), and L-(+)-tartaric acids (HOOC-CHOH-CHOH-COOH, H₂TA) (see Figure 2).

Since H₂DCA species are often stored in grape tissue as potassium salts [1], different H₂DCA potassium salts—prepared by titration of the corresponding acid with KOH (potassium hydroxide)—were used as the starting point in all the MS experiments. In order to investigate the potential role played by counterions, the experiments were also performed on the corresponding sodium and lithium salts (for details see the Supporting Information). DESI analysis was carried out on H₂DCA aqueous solutions deposited and air-dried on a chemically inert porous Teflon surface. For ESI measurements, the same H₂DCA aqueous solutions were directly injected into the ion source using a syringe pump. To minimize possible source-to-source differences, the source geometry of the DESI sprayer was adjusted to serve as the ESI source.

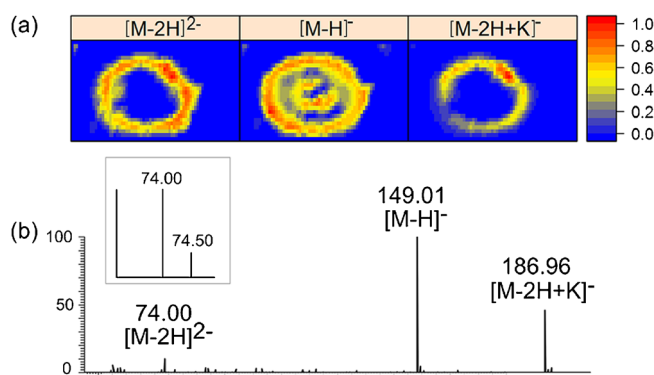


Figure 1. (a) DESI images of tartrate in grapevine leaf petiole. [M-2H]²⁻ denotes doubly charged tartrate dianions (TA²⁻), [M-H]⁻ singly deprotonated ion (HTA⁻), and [M-2H+K]⁻ potassium adduct ions (KTA⁻). (b) Representative spectrum of tartrate in grapevine leaf petiole. The insert shows the half-integer isotope spacing between the ions with molecular formulas ¹²C₄H₄O₆²⁻ at *m/z* 74.00 and ¹²C₃¹³CH₄O₆²⁻ at *m/z* 74.50

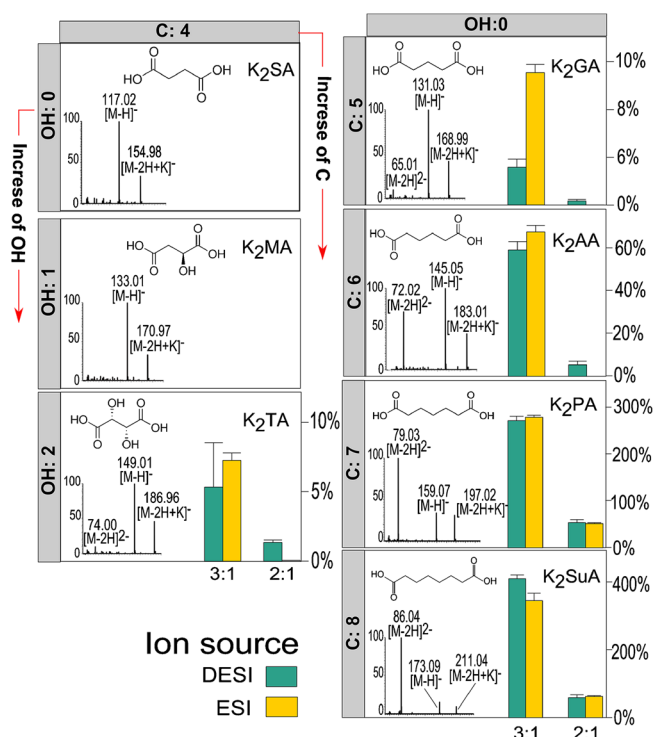


Figure 2. DCA²⁻ ion yields as observed in ESI and DESI ion sources for K₂DCA aq. solutions. DCA²⁻ ion yields were calculated as the ratio of [M-2H]²⁻ abundances to those of their respective [M-H]⁻ singly-charged ions. Values represent mean ± SD (*n* = 6); ‘3:1’ represents salts obtained from H₂DCA using three molar equivalents (3:1) of KOH, whereas ‘2:1’ represents salts obtained from H₂DCA using two molar equivalents (2:1) of KOH. The representative mass spectra shown in the plots are DESI spectra at ‘3:1’. Results for H₂TrA are not shown here as no detectable signal for TrA²⁻ was found either in DESI or in ESI spectra

Experimental

Acetonitrile was purchased from Sigma-Aldrich (Milano, Italy), water was purified using a Mili-Q water purification system (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Succinic (>99.5%), malic (>99.5%), glutaric (>99.0%), and adipic acids (>99.5%) were purchased from Fluka AG Buch, Switzerland; tartronic (97%), L-(+)-tartaric (99.5%), pimelic (98%), suberic acids (98%), lithium hydroxide monohydrate (99%), sodium hydroxide (>97%), and potassium hydroxide (>85%) were from Sigma Aldrich.

The dicarboxylate salts were produced by mixing equal volumes (20 mL, water/acetonitrile 1:4) of 0.5 mM aqueous solutions of each H₂DCA (tartronic, succinic, malic, tartaric, glutaric, adipic, pimelic, and suberic acids) (a) with 3 molar equivalent (1.5 mM) of alkali hydroxides (LiOH, NaOH, KOH) or (b) with 2 mol equivalent (1.0 mM) of the same hydroxides. Three mL of each final solution was deposited on a porous PTFE sheet (pore size 1–3 μm, porosity 25%–30%, Porex Corporation, Bautzen, Germany) and then air dried

before DESI analysis. Each solution was analyzed six times with an acquisition time of 1 min.

All the MS experiments were performed using a Thermo-Fisher Scientific LTQ-Orbitrap XL mass spectrometer (Bremen, Germany) equipped with an OmniSpray ion source from Prosolia Inc. (Indianapolis, IN, USA). The typical instrumental parameters for standard DESI were: -100 V tube lens voltage, -10 V capillary voltage, 3 kV spray voltage, 200°C capillary temperature, 2 micro scans, 200 ms MS injection time. Mass spectra were acquired in full scan negative mode over the m/z range of 50 – 250 . The DESI source parameters used were: 55° spray angle, 1 – 1.5 mm tip-to-surface distance, ~ 4 mm tip-to-inlet distance, 10° collection angle, 8 bar nitrogen carrier gas pressure. A mixture of Milli-Q water and acetonitrile ($1:4$, vol/vol) was used as spray solvent and delivered at the flow rate of $3 \mu\text{L min}^{-1}$.

ESI measurements were carried out by simply changing the DESI spray angle close to 45° , while keeping other instrumental and ion source parameters constant. Analyte solutions were injected directly into the inlet at the flow rate of $3 \mu\text{L min}^{-1}$. Each solution was analyzed six times with an acquisition time of 1 min; ion source was washed after each analysis by spraying Milli-Q water/acetonitrile $1:4$ (v/v) for about 10 min at the flow-rate of $8 \mu\text{L min}^{-1}$.

Results and Discussion

The outcome of our complete set of experiments is summarized in Figure 2, which shows the intensity of the DCA^{2-} peak compared with those of their respective $[\text{M} - \text{H}]^-$ singly-charged ions. Only the results for K_2DCA salts are shown, but similar results were obtained for the corresponding sodium and lithium salts (see Supporting Information Figure S1 for Na_2DCA and Figure S2 for Li_2DCA salts).

Each panel of the plot displays the results relative to H_2DCA species with different carbon backbones (second column) and for the C4 acids bearing different numbers of hydroxyl groups (first column). The bar plots illustrate the results obtained with aqueous solutions prepared from different $\text{KOH}/\text{H}_2\text{DCA}$ ratios. Regardless of the ionization method or $\text{KOH}/\text{H}_2\text{DCA}$ ratio, DCA^{2-} starts to be detectable for a carbon chain of at least five carbon atoms and, as expected, the relative intensity increases with the number of carbons. In ESI/DESI measurements carried out at different $\text{KOH}/\text{H}_2\text{DCA}$ ratios on tartronic acid (HOOC-CHOH-COOH), the only 3-C dicarboxylic acid expected to give stable dianions, no detectable signal for the corresponding dianion was observed.

A longer chain means a greater separation between the two charges and the subsequent reduction of Coulomb repulsion stabilizes the doubly charged ion. Our findings are in keeping with previous investigations, which have shown that DCA^{2-} of $\text{HOOC-(CH}_2)_2\text{-COOH}$ is not stable in the gas phase [6, 14]. A simple calculation of the Coulomb repulsion energy $E_{\text{rep}} = e^2/4\pi\epsilon_0 r$ between two electrons at distance r suggests that the two negative charges in a molecule with a mutual electron affinity of 3 eV (the expected value of carboxylate radical anions) may

be stable when they are more than 5 \AA apart [7]. The charge separation distance of 5.3 \AA for succinate estimated by Wang and collaborators [5] explains why the bare succinate dianion is not observable in the gas phase. In our conditions, the increase in relative intensity with increased length of chain is remarkable: for $\text{HOOC-(CH}_2)_6\text{-COOH}$, the area of the DCA^{2-} species is significantly higher (four times) than that of the singly charged ion HDCA^- . ESI and DESI seem to give qualitatively comparable results, although in ESI the relative yield was found to be slightly higher for glutaric acid (H_2GA).

The effect of the hydroxyl groups on the four-carbon backbone is displayed in the first column of Figure 2. Only H_2TA displays efficient production of dianions in both DESI and ESI. Again, this result is in agreement with the experiments and ab initio calculations reported recently [7], which showed that TA^{2-} is the smallest stable dicarboxylate in the gas phase. Interestingly, in our experiments we were able to obtain a much stronger signal for the TA^{2-} (m/z 74, around 8% of HTA^-) than that reported in recent literature [7], where this species was barely detected, only through measurements carried out under highly restricted ESI conditions.

The strong yield observed here for the formation of TA^{2-} species is difficult to explain. We only note that ESI/DESI yield of this dianion is higher in strongly alkaline solutions as obtained by using a molar excess of metal-hydroxide (MOH). These conditions may give rise to two contrasting phenomena. On one hand, a strongly basic medium is required in order to favor the production of a high molar fraction of TA^{2-} species in the ESI/DESI conditions since the $\text{pK}_{\text{a}1}$ and $\text{pK}_{\text{a}2}$ of H_2TA in acetonitrile/water are expected to be much higher than those in water. On the other hand, in a strongly basic medium the presence of higher concentration of the metal cation M^+ is expected to afford an overall depletion of TA^{2-} species. In fact, the increase of M^+ clearly promotes more efficient ion-pair aggregation leading to the formation of neutrals ($\text{M}^+ - \text{TA}^{2-} - \text{M}^+$) or singly charged species ($\text{TA}^{2-} - \text{M}^+$) during the ESI/DESI processes. The experimental results suggest that, in our conditions, the formation of these ion-pairs is not very efficient. As a possible interpretation of the observed phenomenon, we can hypothesize that during the transfer to the gas phase the metal ions can be selectively and efficiently “caged” by the stronger interaction with OH^- in molar excess in the solution (ESI), making it possible for the DCA^{2-} to eventually reach the gas phase (Figure 3a).

To confirm the role of the free OH^- in solution, we reduced its amount by using only two molar equivalent of MOH to prepare the initial solution. The results of the experiments under these conditions are summarized in the right bar plot in Figure 2 (2:1). The effect of reducing free OH^- on the ESI spectra is striking: DCA^{2-} is practically undetectable in all ESI cases, at least for H_2DCA species with low carbon atoms ($C \leq 6$). A minor signal is still visible (0.075% for H_2TA , 0.006% for H_2AA), but it is akin to chemical noise and it is in line with what is reported in the literature [7]. Weak signals attributable to PA^{2-} and SuA^{2-} were nevertheless found in this condition, because of the longer distance between the two carboxylated groups of pimelic and suberic acids. This experiment unambiguously confirms the role of OH^- for enhancing the yield of

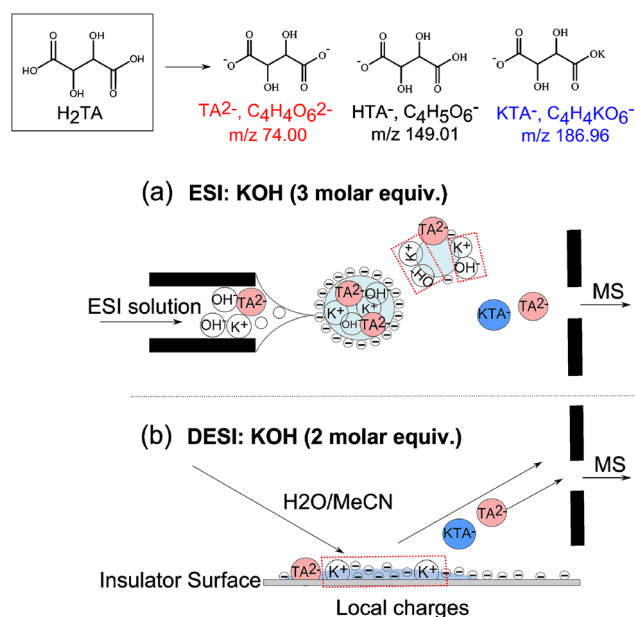


Figure 3. The proposed mechanisms of dianion production in ESI and DESI. In ESI, some of the K⁺ ions are “caged” by the presence of one excess free OH⁻ during ionization, and they are not available to completely neutralize the most abundant DCA²⁻ species in highly alkaline conditions. In DESI, even when there is no excess free OH⁻, the negative charges accumulated on the surface also “cage” K⁺ ions during desorption, thereby favoring the release of free DCA²⁻ ions in the gas phase

dianions species, possibly by reducing the extent of ion pairing processes in ESI conditions.

In the DESI measurements on these H₂DCA 2:1 solutions the results, however, were different. Although the DCA²⁻ yield was lower than the one observed for H₂DCA 3:1 solutions, the signals were still clearly present. A straightforward way to account for this observation is to suppose that in DESI there is another active “caging” mechanism that mimics the effects of the free OH⁻ excess. The particular nature of DESI suggests a possible solution. It is known that DESI is a two-step process where the initial desorption of the analytes is followed by their ionization in an ESI-like process. The first step involves the landing and releasing of charges on the surface [15], and when the surface is an insulator, as in our case, this will produce a local charge accumulation [16]. In negative ion mode, the negatively charged surface can sequester the K⁺ ions favoring the release of free DCA²⁻ ions in the gas phase (Figure 3b).

Conclusions

We have demonstrated a simple and efficient method for producing good yields of doubly charged dicarboxylates (in particular for small H₂DCA such as tartaric, glutaric, and adipic acids) from their alkaline H₂O/CH₃CN solutions under standard DESI and ESI conditions.

Our DESI results were obtained on a PTFE model surface, but the same phenomena are expected to occur on plant tissue

sections, where the large amount of OH-rich polymers is expected to bind metal ions, favoring the formation of the DCA²⁻ observed during our imaging experiments. From an analytical point of view, our study suggests that DCA²⁻ signal could be used in DESI imaging experiments as a proxy to measure the distribution of H₂DCA salts in plant tissues. The potential application of this idea will be explored in further investigations.

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