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Adduct formation in electrospray ionization mass spectrometry II. Benzoic acid derivatives

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Abstract

This work serves as a follow-up to Part I of experiments designed to determine the underlying principles in the formation of pseudomolecular, or adduct, ions during electrospray ionization. Aromatic acids were studied by flow injection analysis in the negative ionization mode of electrospray ionization mass spectrometry. Part I dealt with common acidic anti-inflammatory pharmaceuticals, such as ibuprofen and related analogues. Part II deals with functionally less complex molecules, namely benzoic acid (BA) and substituted benzoic acids. Halide-substituted molecules are investigated to deduce the effect of electron-withdrawing substituents (bromo-, chloro-, and fluoro-) and ring position (*ortho*-, *meta*- and *para*-) on the response of a traditional deprotonated molecular ion ($[M-H]^-$) and a sodium-bridged dimer ion ($[2M-2\ H+Na]^-$). Amino-substituted benzoic acids are also analyzed in order to study the effect of an additional ionizable group on the molecule, and *para-tert*.-butyl-BA is analyzed to study the effect of increased hydrophobicity, as they relate to the formation of pseudomolecular ions. This study shows that solution character [octanol–water partition coefficient (or log P) and pK_a] of the model compounds controls the relative efficiency of formation of $[M-H]^-$ and $[2M-2H+Na]^-$ ions. However the relative gas phase character (gas phase basicity and proton affinity) also has a significant effect on the formation of the sodium-bridged dimer ion. For the halide-substituted species, placement of the electron-withdrawing atom at the *meta*-position gives the greatest enhancement in sensitivity. Observations also show that as the structural complexity of the model compound increases, predictions relating analyte acidity to sodium-bridged dimer ion formation give way to a stronger dependence between log P values and ionization efficiency. Supporting this hypothesis is the nearly ten-fold enhancement in signal for *tert*.-butyl BA relative to BA, due to the greater hydrophobicity, and consequently, increased surface activity in an electrosprayed droplet of the analyte molecule.

Keywords: Mass spectrometry; Adduct ions; Benzoic acid

1. Introduction

Electrospray ionization mass spectrometry (ESI-MS), particularly in combination with liquid chromatography (LC), has become widely used in the past twenty years as a versatile tool for converting solution-phase species into gas-phase ions. The soft nature of the ESI process generates spectra where molecular ions are largely intact with little fragmentation. As a result, both advantages and disadvantages are realized. ESI has been used recently for studies of noncovalent solution and gas-phase association of species, particularly in the realm of molecular recognition, metal chelation, and complex formation in general [1–13]. Also well known is the capability of ESI-MS for the analysis of high-molec-

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ular-mass polymers and proteins due to multiple charging [14]. This technique however has a number of drawbacks: (1) the large array of LC mobile phase systems makes ESI spectra largely unpredictable and as a result, libraries are not available without strict control of the mobile phase composition; (2) pseudomolecular ions, also commonly referred to as adduct ions, are produced between analytes and other solution species that can complicate qualitative analysis and spectral interpretation and (3) ESI-MS is applicable only to polar and ionic analytes in solution, limiting to a degree the range of possible applications.

This study focuses on the formation of pseudomolecular ions by ESI-MS, particularly those formed by aromatic acid groups in negative electrospray ionization. The work presented here is a follow-up to Part I of this study which focused on negative mode ESI-MS of six common acidic pharmaceuticals, specifically ibuprofen, ketoprofen, naproxen, flurbiprofen, fenoprofen, and carprofen [15]. Those molecules all contain a phenyl acetic acid moiety and differ from each other in their substituent arrangement. Results of Part I experiments showed that, even though five out of six of the model compounds formed intense deprotonated molecular ions ($[M-H]^-$), the range of abundances of the deprotonated molecular ion, as well as the two pseudomolecular ions, a homogeneous dimer ion ($[2M-H]^-$) and a deprotonated dimer ion pair with sodium ($[2M-2H+Na]^-$), varied greatly for the six different analytes. In fact, only general qualitative trends could be established, owing to the functional complexity of the molecules. These trends are briefly summarized as follows:

(i) In the absence of sodium salts, deprotonated molecular ions of most organic acids can be observed in the negative ionization mode;
(ii) Little to no ions corresponding to aromatic acid analytes are observed in the positive ionization mode;
(iii) Homogeneous dimer ion formation is readily observed when hydrogen bonding groups are present in aromatic acids;
(iv) All of the aromatic acid molecules studied complex with background Na^+ ions to form stable dimer ion pair adducts.

The complexity of functionality of the ibuprofen derivatives warranted a simpler study. The work reported here focuses on a set of model compounds encompassing benzoic acid and substituted benzoic acid (BA) molecules. Fig. 1 shows the structures of the benzoic acid derivatives selected to study substituent effects on the intensity of pseudomolecular ion formation. By substituting a range of electron-withdrawing groups on BA (in this study bromine, chlorine, and fluorine were used), acidity and solubility of the molecule are altered based on the ring position and electronegativity of the substituent. Amine and alkyl substituted BA model compounds are also included to study systems with increased functionality and increased hydrophobicity, respectively. These effects are studied by monitoring ion response using flow injection analysis (FIA)–ESI-MS as described in Part I [15].

Also included in this study are a series of experiments characterizing the response of unsubstituted BA under various instrument conditions. Varying the percent acetonitrile in an acetonitrile–water system allows an indirect study of ionization of BA relating to droplet formation, specifically the effect of the solvent dielectric on sodium-bridged dimer ion formation and the surface activity of BA in deprotonated molecular ion formation. Interface conditions between the LC and MS were also studied, specifically the voltage and temperature of the curved desolvation line (CDL). The CDL is the inlet to the mass spectrometer which separates the atmospheric pressure and high vacuum regions of the instrument and is the pathway for extraction of the ions into the mass spectrometer. The effect of the CDL voltage is akin to in-source collision induced dissociation, where a higher voltage will influence ionization of molecules in the gas-phase by initiating a greater number of collision reactions. Often, this parameter

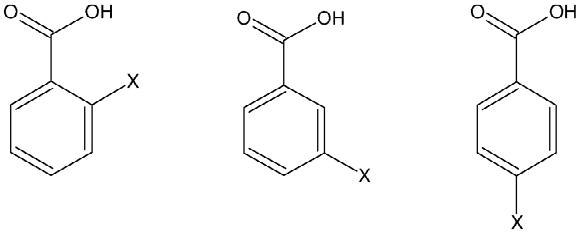

Fig. 1. Structures of halide-substituted benzoic acid model compounds (X = Br, Cl, and F).

may be used to study fragmentation of molecules, however the ion selectivity of the in-source induced collision reactions is poor in comparison to modern tandem MS instrumentation. The CDL temperature is also varied to study the effect on ion formation. The heat applied to the CDL serves to decluster analyte–solvent clusters sampled from the gas phase. Thermally labile species are susceptible to decomposition when the CDL temperature is too high and inefficient declustering of gas-phase species (low ionization) can result in if the CDL temperature is too low.

The effect of instrument parameters on unsubstituted BA ion formation serves as a basis for establishing trends related to substituent effects as well as a means to characterize optimum operating conditions for ionization of aromatic acids. Results from these experiments serve as building blocks for elucidating pseudomolecular ion formation for species with higher functionality, such as the ibuprofen derivatives previously studied [15]. Comparison of ionization trends to tabulated and calculated solution-phase and gas-phase physical chemical data are used to deduce the point of formation of particular pseudomolecular ions (solution-phase versus gas-phase) as well as the relative importance of each physical parameter in influencing the negative mode ionization of aromatic acids.

2. Experimental

The instrument used was the Shimadzu LCMS-2010 API mass spectrometer (Shimadzu Scientific Instruments, Columbia, MD, USA). Standard LC-10AD VP dual-piston pumps with high pressure mixing provided flow for the interface and detector. The pumps were operated isocratically at 0.1 ml/min with a mobile phase of acetonitrile–water (50:50) for work with substituted BA analytes and acetonitrile–water 1:99, 25:75, 50:50, and 75:25 for studies of BA in different mobile phase compositions. The acetonitrile used was HPLC grade (Burdick and Jackson, Muskegon, MI, USA) and the deionized water was obtained in-house (pH 6.0) and filtered prior to use. Samples were prepared in standard autosampler vials for flow injection analysis. A 200-μl volume of 0.01 m*M* samples was injected by the Shimadzu SIL-10AD VP autosampler. The effect of changing concentration of analyte was also investigated for BA. *meta*-Bromobenzoic acid (3-Br-BA), *meta*-chlorobenzoic acid (3-Cl-BA), *para*-chlorobenzoic acid (4-Cl-BA), *meta*-aminobenzoic acid (3-amino-BA), and *para*-aminobenzoic acid (4-amino-BA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). *ortho*-Bromobenzoic acid (2-Br-BA), *para*-bromobenzoic acid (4-Br-BA), *ortho*-chlorobenzoic acid (2-Cl-BA), *ortho*-fluorobenzoic acid (2-F-BA), *meta*-fluorobenzoic acid (3-F-BA), *para*-fluorobenzoic acid (4-F-BA), and *para-tert.*-butylbenzoic acid (4-*tert.*-butyl-BA) were obtained from Lancaster (Pelham, NH, USA). *ortho*-Aminobenzoic acid (2-amino-BA) was obtained from Acros Organics (Fisher, Geel, Belgium).

The LCMS-2010 was operated in the negative ionization ESI scan and the selected-ion monitoring (SIM) modes. Scans were made from 50 to 500 at 0.5-s intervals (scan speed=1000 amu/s). SIM was used for unsubstituted BA experiments at m/z 121 ($[M-H]^-$) and m/z 265 ($[2M-2H+Na]^-$). The temperature and voltage of the curved desolvation line (the inlet for the high vacuum region) were set to 250 °C and −25 V, respectively, for analyses of the substituted BA compounds. For all experiments, the probe voltage and nitrogen nebulizer gas flow remained constant at −3.5 kV and 4.5 l/min, respectively. These operating conditions are mild enough to preserve association between solution-phase species and thus, pseudomolecular ions can be observed in the ESI mass spectra. The CDL temperature and voltage values were varied appropriately for BA experiments focused on studying the effect of these parameters on sampling efficiency and consequently, ion formation.

Flow injection analysis of the compounds is used as a quick and easy way to study pseudomolecular ion formation by ESI-MS. In this configuration, no column is employed and the LC system is simply a sample introduction apparatus. For reliable data, certain criteria must be considered when establishing the experimental method for FIA–ESI-MS. As previously stated, 200 μl injections were made at a flow-rate of 0.1 ml/min. Each analyte was analyzed individually under the prescribed conditions. Assuming no band broadening, the sample injection band is infused into the interface for two min. In this time, an equilibrium, or quasiequilibrium, is established in

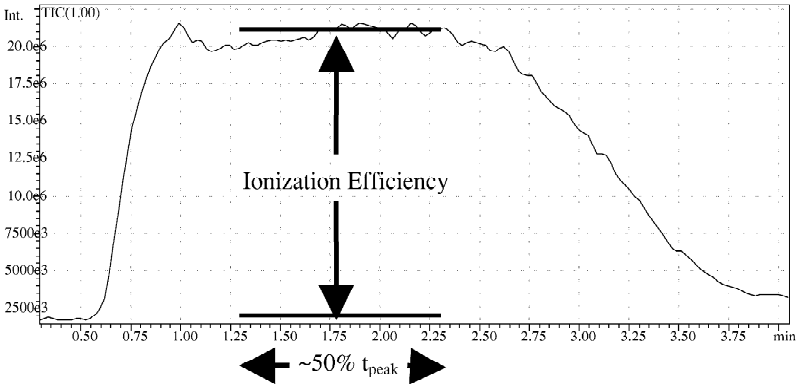

Fig. 2. Total ion chromatogram for FIA-ESI-MS of carprofen; 200 μl of sample was infused into the source at 0.1 ml/min. Ionization efficiency is depicted as the average of the ion intensity over a set of quadrupole scans taken during the plateau or quasiequilibrium time frame.

the source chamber and the result is a plateau response, where the average maximum of this response plateau is the relative ionization efficiency for an analyte. Fig. 2 depicts a common FIA–ESI-MS large volume injection profile. The value obtained for intensity of an ion is determined from the average of ~50% (centroid±25%) of the scans taken in the quasiequilibrium plateau region. For this work, a 500-μl sample loop was placed in the autosampler. As a result of the extra volume in the loop and the large injection volume, broad bands of analytes were ionized allowing a large number of peak scans.

3. Results and discussion

The first set of experiments studied the effect of mobile phase composition and ESI source parameters on the formation of benzoic acid pseudomolecular ions. For this study, 40 samples were prepared at eight concentrations in the presence of five different mobile phase compositions. Benzoic acid was prepared at 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001, $1 \cdot 10^{-5}$, and $1 \cdot 10^{-6}$ m*M* concentration levels in each mobile phase: acetonitrile–water (1:99) (25:75); (50:50); (75:25) and (99:1). Each sample was run at normal LC–MS tune parameters (CDL temperature = 250 °C, CDL voltage = −25 V). In addition, BA was analyzed with CDL voltages of −10, −70, −120 and −180 V. The CDL temperatures investigated were 200, 250 and 300 °C. The results of each experiment are shown in Figs. 3 and 4.

Fig. 3 shows the response of the deprotonated molecular ion for benzoic acid at different concentrations in the presence of changing mobile phase composition. As expected, ionization efficiency increases dramatically with an increase in percent organic mobile phase. A high percentage of organic allows for the formation of smaller droplets due to the decreased surface tension. This increases the ionization efficiency, allowing a greater number of ions to evaporate into the gas-phase. The results for 99% acetonitrile are not included here due to the

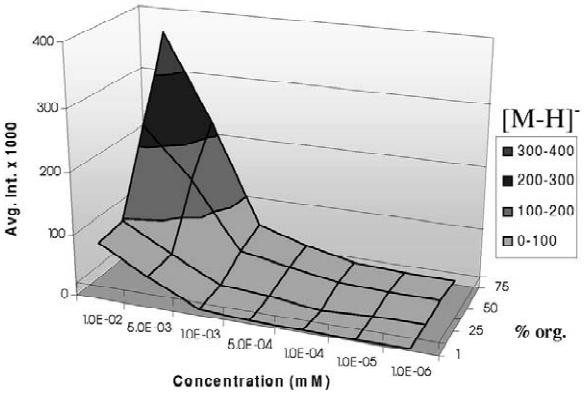

Fig. 3. Three-dimensional plot relating response of benzoic acid deprotonated molecular ion ($[M-H]^-$) to percent organic (acetonitrile in an acetonitrile–water system) as a function of concentration. Note in this figure that concentration increases from right to left for the purpose of visualizing the data.

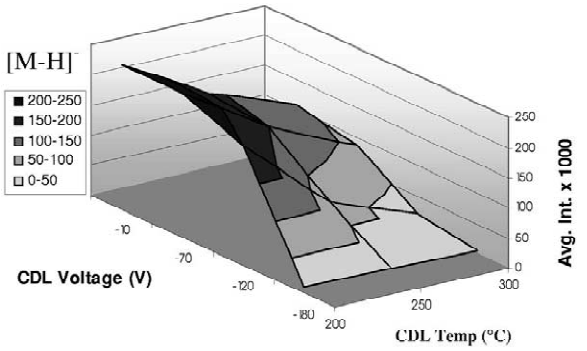

Fig. 4. Effect of CDL voltage and temperature on the response of benzoic acid deprotonated molecular ion ($[M-H]^-$). Benzoic acid was present at 0.01 m*M* concentration.

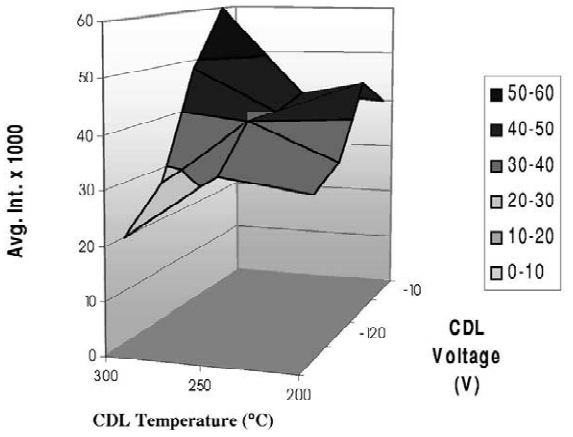

Fig. 5. Effect of CDL voltage and temperature on response of the benzoic acid sodium-bridged dimer ion ($[2M-2H+Na]^-$). The response of this pseudomolecular ion is greatest at CDL temperature = 300 °C and CDL voltage = −10 V.

erratic response at this high organic concentration. Though intensity increases as percent organic increases, a certain amount of water must be present to provide adequate conduction in the droplets. At the other extreme, poor response is observed at low concentration of organic due to the large droplets formed by the high surface tension of water. Also hindering ionization is the low vapor pressure of water relative to acetonitrile, which makes droplet evaporation more difficult. These studies show that there exist optimum conditions for ionization of BA in acetonitrile–water solution systems. The results suggest a lower limit of detection of BA in a mobile phase containing a high percentage of organic solvent.

Fig. 4 shows the effect of MS inlet parameters on response of the deprotonated molecular ion for BA. For this study, 0.01 m*M* BA was analyzed while changing CDL voltages and temperatures. The graph depicts the thermal lability of $[M-H]^-$ for BA in response to increasing CDL temperature. Signal is diminished by almost a factor of two at 300 °C versus 200 °C. Also shown is the lack of stability of the $[M-H]^-$ ion with respect to increased collisional interactions at high CDL voltages. Signal begins to dramatically decrease above −100 V and goes to zero when the CDL voltage is set to −180 V. This study shows the relative stabilities of the BA deprotonated molecular ion with respect to temperature and collisional decomposition and provides guidelines for increasing ionization efficiency. As a comparison, the same plot is made for the response of the sodium-bridged dimer ion in Fig. 5. Notable is the exceptional stability of the $[2M-2H+Na]^-$ ion in the presence of higher temperatures and voltages. This confirms the greater stability of the sodium-bridged dimer ion and helps explain the observation of this pseudomolecular ion for all aromatic acid molecules studied thus far.

All substituted BA sample analyses were performed under normal tune conditions in acetonitrile–water (50:50). Positions of the ESI probe and other MS parameters were kept constant to provide a better basis for comparison.

The next sets of experiments were designed to study the effects of substituents on pseudomolecular ion formation. Bromine, chlorine, and fluorine, at different ring positions, were used to evaluate the effect of electron-withdrawing groups on pseudomolecular ion intensity. Also included in this study were amino-substituted benzoic acids to investigate the response when a second ionizable functional group is present. *para-tert.*-Butylbenzoic acid was analyzed to study the effect of increased hydrophobicity as well as increased steric bulk.

Solution-phase data calculated for all of the analytes studied are shown in Table 1. These were calculated using the Advanced Chemistry Development (ACD Labs., Toronto, Canada) SPEC MANAGER pK_a and log *P* calculators. Log *P* is the common nomenclature for the logarithm of an octanol–water

Table 1
Solution phase data for benzoic acid and substituted benzoic acid model compounds[a]

Analyte	pK_a[a]	log P[a]
Benzoic acid (BA)	4.2	1.89
4-F-BA	4.14	2.07
4-Cl-BA	3.97	2.65
4-Br-BA	3.97	2.86
3-F-BA	3.86	2.16
3-Br-BA	3.81	2.71
3-Cl-BA	3.38	2.9
2-F-BA	3.27	1.86
2-Cl-BA	2.97	2.04
2-Br-BA	2.85	2.15
2-Amino-BA	4.94	1.21
3-Amino-BA	4.75	0.78
4-Amino-BA	4.86	0.001
4-*t*-Butyl-BA	4.4	3.58

[a] Values calculated from Advanced Chemistry Development, SPEC MANAGER, ACD, Toronto, Canada.

partition coefficient. The values show the relative effect of each substituent on the acidity and hydrophobicity of the modified benzoic acid. Addition of electronegative groups makes the molecule more acidic and slightly more hydrophobic relative to benzoic acid. Intuitively, the addition of an electron-withdrawing group would make a molecule more ionic (e.g. less hydrophobic), however the calculated log P values show that the halide substituent actually increases the affinity of the molecule for a hydrophobic phase boundary. This is further supported by the increase in ionization efficiency for the halide BA molecules relative to unsubstituted BA.

Table 2 shows the response of $[M-H]^-$ for the halide substituted benzoic acids. An increase in log P causes an increase in the ionization of the deprotonated molecular ion. This is expected, since the molecule will have a higher surface activity in an aqueous droplet and will migrate faster to the droplet/air interface, thus increasing efficiency for ion evaporation. This relationship has been reported previously by Cech and Enke in their work detailing the effect of increased ESI response with increased length of nonpolar side chain on small peptide molecules [16]. In all cases, the *meta* position is the most active for promoting ionization efficiency of the halide-substituted benzoic acid pseudomolecular ions.

Table 2
Average intensity of the $[M-H]^-$ and $[2M-2H+Na]^-$ pseudomolecular ions for halide-substituted benzoic acids in acetonitrile–water (50:50); all analytes were present at 0.01 m*M* concentration

Analyte	Average intensity/1000	
	$[M-H]-$	$[2M-2H+Na]-$
o-Br-BA	410	60
o-Cl-BA	430	71
o-F-BA	330	74
m-Br-BA	500	110
m-Cl-BA	550	130
m-F-BA	540	200
p-Br-BA	430	98
p-Cl-BA	470	130
p-F-BA	420	160

Also shown in Table 2 is the response of the dimer ion pair ($[2M-2H+Na]^-$) for the halide-substituted benzoic acids. These data correlate best with changes in pK_a associated with electronegative groups. Molecules with lower pK_a values form higher intensity dimer ion pair ions relative to BA. This is rationalized by the greater availability of coulombic sites for background Na^+ ion to associate with the deprotonated acid molecules. However, within a set of halide-substituted BA compounds (e.g. *ortho*-, *meta*-, and *para*-chloro-BA), the intensity of $[2M-2H+Na]^-$ increases with pK_a. In other words, *meta*- and *para*-chloro-BA have a higher intensity despite being less acidic. This trend holds for all of the halide-substituted BA models and may be explained by the steric hindrance in the *ortho*-position which interferes with the approach and complex formation by Na^+.

Amino- and *tert*.-butyl-substituted benzoic acids were also analyzed under the same conditions as the halide-substituted compounds (0.01 m*M* concentration in acetonitrile–water, 50:50). The results for the $[M-H]^-$ and $[2M-2H+Na]^-$ average ion abundances are shown in Table 3. *tert*.-Butyl-BA with a much higher log P is more hydrophobic, and consequently, with higher droplet surface activity, the ion abundance of the deprotonated molecular ion is enhanced nearly ten-fold compared to unsubstituted BA. The amino-BA data are not as easy to interpret. The amino-substituent causes an increase in pK_a which should increase sodium-bridged dimer ion formation; however the effect of ring position on pK_a

Table 3
Pseudomolecular ion average intensity for amino- and *p-tert.*-butyl-substituted BA analytes; all analytes were present at 0.01 m*M* concentration

Analyte	Average intensity/1000	
	$[M-H]^-$	$[2M-2H+Na]^-$
BA	290	63
o-Amino-BA	340	130
m-Amino-BA	240	60
p-Amino-BA	190	30
tert.-Butyl-BA	1200	140

is minimal whereas its effect on $[2M-2H+Na]^-$ response is significant.

A greater effect of substituent position is shown for *ortho*-, *meta*- and *para*-amino benzoic acid. As with the halide-substituted BA model compounds, the response of the amino-substituted deprotonated molecular ion ($[M-H]^-$) follows the trend of log *P* values (e.g. *ortho*-amino-BA has the highest log *P* value and the highest response whereas *para*-amino-BA has the lowest log *P* value and the lowest response). The response of the sodium-bridged dimer ion for amino-substituted BA does not conform to the trend observed with halide-substituted BA where increased ionization is observed with increased pK_a. Instead, response of $[2M-2H+Na]^-$ is most intense for the *ortho*-amino substituted species and is least intense for the *para*-amino substituted species; the same trend that is observed for the molecular ion. In the case of the sodium-bridged dimer ion formation for *ortho*-amino-BA, the increase in response, relative to substitution at other ring positions, is most likely due to resonance stabilization effects. These data show that the increase in complexity of a molecule with increased functionality makes predictions of ionization response for pseudomolecular ions from solution-phase data more difficult.

Table 4 lists gas-phase physical chemical data for the proton affinity and gas-phase basicity of a few common molecules [17]. Although data for most of the molecules used in this study were not available, benzoic acid is included as well as the effect of electronegative substituents on these parameters for other species. The gas-phase basicity (GB) and proton affinity (PA) of a molecule characterizes its behavior in the gas-phase. Simply defined, the GB is

Table 4
Gas-phase basicities and proton affinities for selected analytes [17]; values for benzoic acid, acetonitrile, and water are italicized; values for the substituted benzoic acids were not available in the literature

Analyte	Gas-phase basicity (kcal/mol)	Proton affinity (kcal/mol)
Triethylamine	224.5	232.3
Formaldehyde	221	229
Aniline	202.5	209.5
3-Iodoaniline	201.1	208.9
4-Chloroaniline	201	208.6
3-Bromoaniline	200.3	208.1
4-Fluoroaniline	200.3	208.1
3-Chloroaniline	199.4	207.2
3-Fluoroaniline	199.2	207
Benzaldehyde	192.4	200.2
4-Chlorobenzaldehyde	192.4	200.2
4-Fluorobenzaldehyde	191.4	199.1
Benzoic acid	*189.6*	*198.2*
3-Fluorobenzaldehyde	188.7	196.5
Acetic acid	181.7	190.2
Acetonitrile	*180.6*	*188.4*
Ethanol	180.2	188.3
Methanol	174.1	181.9
Water	*159*	*166.5*
Nitrogen gas	111	118.2

1 cal = 4.184 J.

$-\Delta G_{rxn}$ and the PA is $-\Delta H_{rxn}$ for a simple gas-phase protonation reaction: $M+H^+ \rightarrow MH^+$.

The values in Table 4 allow deductions concerning the relative gas-phase behavior of benzoic acid in our system as well as the effect of halide substitution on shifts in values for GB and PA in other structurally similar model compound sets (e.g. benzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, etc.). The first point to note is that the PA and the GB of benzoic acid is higher than both acetonitrile and water. If sodium-bridged dimer ion species are formed in the gas phase and if we assume that PA is applicable to association of species with sodium ion, then it follows that benzoic acid will have a greater affinity for sodium ion than both water and acetonitrile and adduct ions will be observed. This hypothesis is consistent with observations made in our experiments.

The next point to note is the shift in GB and PA values with halide substitutions. Included in Table 4 are values for aniline and aniline derivatives, as well

as, benzaldehyde and benzaldehyde derivatives. In both cases, the PA and GB of halide-substituted species are slightly lower than the PA and GB of unsubstituted species. As a consequence, it is expected that halide-substitution would cause a decrease in the affinity of the molecule for sodium ion in the gas-phase. This does not agree with the data shown in this study and suggests that the point of association of pseudomolecular ions, specifically sodium-bridged dimer ions of aromatic acids, is in the solution phase rather than through collisional association in the gas phase. To confirm this hypothesis, it will be necessary to perform a similar study to what is presented in the paper, except using a model compound set, such as benzaldehyde and halide-substituted benzaldehyde, where gas-phase physical chemical data are already well known.

4. Conclusions

The results presented here show an in-depth study on the effect of substituents on the formation of aromatic acid pseudomolecular ions, specifically a deprotonated molecular ion ($[M-H]^-$) and a sodium-bridged dimer ion ($[2M-2H+Na]^-$), by negative ionization mode FIA–ESI-MS. The model compound set consists of BA and halide-, amino-, and alkyl-substituted BA molecules. ESI-MS responses of these ions are compared to solution-phase data, such as pK_a and log *P*, as well as to gas-phase data, such as GB and PA to deduce the point of formation of pseudomolecular ions.

Benzoic acid is a small aromatic acid consisting of a single ionic functional unit. By varying the solution environment as well as instrumental parameters, it is apparent that BA is a thermally labile molecule that ionizes best under soft conditions where a high percentage of organic in an organic–aqueous system is present and very little collisional energy is imparted by the CDL, or MS inlet. Substitutions to BA generally cause an increase in ionization (or an increase in the stability of the ion after formation) of the molecule relative to unsubstituted BA.

The halide-substituted BA model compounds appear to ionize as pseudomolecular ions according to their solution phase character. Trends in ESI-MS response for the $[M-H]^-$ ion closely follow the trend in log *P*, the octanol–water partition coefficient, where an increase in log *P*, or hydrophobicity, causes an increase in the surface activity of the molecule, and consequently, an increase in ionization efficiency. The $[2M-2H+Na]^-$ ion response for the singly substituted aromatic acids more closely mirrors the trend of the pK_a of the molecules. The placement of an electron-withdrawing group on benzoic acid makes it a stronger acid and gives sodium ions a greater opportunity to join dissociated acids in the electrosprayed droplet. The result is an increase in the response of $[2M-2H+Na]^-$ in ESI mass spectra.

Formation of the sodium-bridged dimer ion may also be hypothesized to form through gas-phase collisions, however $[2M-2H+Na]^-$ ionization efficiency seems to be controlled more by solution-phase behavior, such as surface activity and acidity, in the case of the halide-substituted benzoic acids. More than likely, declustering in the gas phase of evaporated ion clusters creates a system where sodium associates with the gas-phase aromatic acids to a much greater degree than to solvent evaporating from the ion cluster. This is due to the greater GB and PA of the benzoic acid, and presumably all aromatic acids, compared to water and acetonitrile, assuming that PA is directly correlated to sodium affinity.

This hypothesis is further correlated with the previous work with ibuprofen derivatives in two ways. First, all of the ibuprofen derivatives, composed mainly of substituted phenyl acetic acid moieties, formed dimer ion pairs with sodium in an acetonitrile–aqueous system. This is consistent with all analyses performed on aromatic acidic molecules by FIA–ESI-MS thus far. Upon addition of triethylamine to the system, there was a general decrease in the response of $[2M-2H+Na]^-$ ions. Triethylamine has a very high PA relative to the other species in the system, and therefore it has a higher affinity for the sodium ions in the decomposing evaporated ion cluster. Secondly, there existed a high degree in variance of dimer ion pair response for the ibuprofen derivative molecules. Some molecules, such as carprofen, showed a higher degree of association with sodium ions than other molecules, such as ibuprofen. Carprofen contains an aromatic amine group and might be expected to have a higher

proton affinity than ibuprofen, which is an alkyl substituted phenyl acetic acid. Consequently, although ibuprofen still registers some signal for the dimer ion pair, the formation of the carprofen dimer ion pair is a stronger association during the decomposition of the ion clusters.

The next consideration in this experiment is the effect of the ring position on ionization efficiency. For all of the halide-substituted benzoic acids, the *meta* position was the most active for increasing ionization efficiency relative to BA, followed by substitution at the *para* position, with substitution at the *ortho* position providing the least enhancement. This suggests that the surface activity, represented by log *P*, has a greater effect on ionization efficiency, than solution phase acidity for the halide-substituted aromatic acid molecules.

The same trend was observed for the effect of the position of amino-substituents. While the substitution of the amine group at different positions on the aromatic ring had little effect on the acidity of the acid group, the observed trend for ionization response as a function of ring position was: *ortho*→*meta*→*para*-amino BA. This trend was observed for the response of both $[M-H]^-$ and $[2M-2H+Na]^-$ and correlated most readily with the trend of calculated log *P* values (see Table 1) for the model compounds.

When hydrophobicity of the molecule is drastically increased, through the substitution of a *para-tert.*-butyl group, there is a drastic increase in response in negative ionization mode ESI-MS. The ionization efficiency of 4-*tert.*-butyl BA is approximately ten times greater than unsubstituted BA. Such an effect was expected and has been reported previously [16]. A comparable example in Part I of this work was the increased ionization efficiency of the deprotonated molecular ion for ibuprofen, due to the increased surface activity imparted by its propyl alkane tail, relative to the other ibuprofen derivatives.

Though there still remain few to no well-defined guidelines for predicting relative responses of pseudomolecular ions in ESI-MS, this work gives insight into the factors contributing to efficient ionization of aromatic acids in the negative ionization mode. Results for smaller aromatic acids have been used to rationalize changes in response for more highly functionalized species based on monitoring pseudomolecular ion response under a variety of common LC–MS conditions. It is apparent that higher functionality complicates prediction, and ultimately, two conclusions may be drawn: (1) formation of aromatic acid pseudomolecular ions, such as $[M-H]^-$ and $[2M-2H+Na]^-$, is largely controlled by the relative surface activity of the analyte; (2) formation of $[2M-2H+Na]^-$ is influenced by the relative PA of species present in a decomposing evaporated ion cluster.

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References

[1] J.M. Daniel, S.D. Friess, S. Rajagopalan, S. Wendt, R. Zenobi, Int. J. Mass Spectrom. 216 (2002) 1.
[2] W.A. Tao, D. Zhang, E.N. Nikolaev, G. Cooks, J. Am. Chem. Soc. 122 (2000) 10598.
[3] J.S. Brodbelt, Int. J. Mass Spectrom. 200 (2000) 57.
[4] S.M. Blair, E.C. Kempen, J.S. Brodbelt, J. Am. Soc. Mass Spectrom. 9 (1998) 1049.
[5] E.N. Nikolaev, E.V. Denisov, V. Sergey Rakov, J.H. Futrell, Int. J. Mass Spectrom. 182/183 (1999) 357.
[6] C.V. Robinson, E.W. Chung, J. Am. Chem. Soc. 118 (1996) 8646.
[7] S.L. De Wall, L.J. Barbour, G.W. Gokel, J. Am. Chem. Soc. 121 (1999) 8405.
[8] M.B. More, D. Ray, P.B. Armentrout, J. Am. Chem. Soc. 121 (1999) 417.
[9] T.J.D. Jørgensen, P. Roepstorff, A.J.R. Heck, Anal. Chem. 70 (1998) 4427.
[10] M. Möder, K. Wichman, K. Gloe, F. Vögtle, Int. J. Mass Spectrom. 210/211 (2001) 327.
[11] J.A. Stone, D. Vukomanovic, Int. J. Mass Spectrom. 210/211 (2001) 341.
[12] T.J.D. Jørgensen, T. Staroske, P. Roepstorff, D.H. Williams, A.J.R. Heck, J. Chem. Soc. Perkin Trans. 2 (1999) 1859.
[13] R. Bakhtiar, H. Chen, S. Ogo, R.H. Fish, Chem. Commun. (1997) 2135.
[14] R.B. Cole, J. Mass Spectrom. 35 (2000) 763.
[15] K. Schug, H.M. McNair, J. Sep. Sci. 25 (2002) 760.
[16] N.B. Cech, C.G. Enke, Anal. Chem. 72 (2000) 2717.
[17] S.G. Lias, J.F. Liebman, R.D. Levin, J. Phys. Chem. Ref. Data 13 (1984) 695.