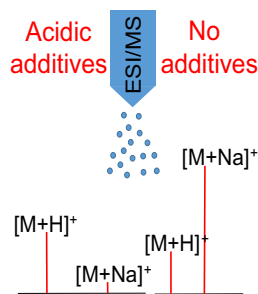**RESEARCH ARTICLE**

Adduct Formation in ESI/MS by Mobile Phase Additives

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Abstract. Adduct formation is a common ionization method in electrospray ionization mass spectrometry (ESI/MS). However, this process is poorly understood and complicated to control. We demonstrate possibilities to control adduct formation via mobile phase additives in ESI positive mode for 17 oxygen and nitrogen bases. Mobile phase additives were found to be a very effective measure for manipulating the formation efficiencies of adducts. An appropriate choice of additive may increase sensitivity by up to three orders of magnitude. In general, sodium adduct $[M + Na]^+$ and protonated molecule $[M + H]^+$ formation efficiencies were found to be in good correlation; however, the former were significantly more influenced by mobile phase properties. Although the highest formation efficiencies for both species were observed in water/acetonitrile mixtures not containing additives, the repeatability of the formation efficiencies was found to be improved by additives. It is concluded that mobile phase additives are powerful, yet not limiting factors, for altering adduct formation.

Keywords: Sodium adducts, Electrospray, Ionization efficiency, Mobile phase additives, Ammonium adducts
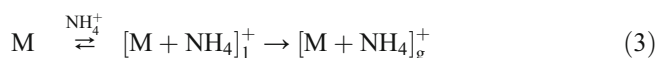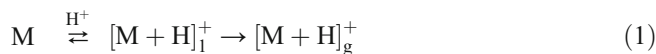
Introduction

Adducts are frequently observed in both the positive and negative modes of electrospray ionization (ESI), although more common in the former [1]. For compounds that cannot be directly analyzed with electrospray ionization mass spectrometry (ESI/MS), such as sugars and various explosives [2], adduct formation has been found to be very useful. For example, Ghosh and Jones [3] achieved determination of acylsucrose metabolites via formate adducts in ESI negative mode despite the fact that these compounds do not contain ionizable sites. Also, the determination of small anions present in samples with very low concentrations has been achieved with paired-ion ESI, where the target anions form adducts with large multiply charged cations [4].

In addition to the commonly observed protonated molecules, sodium, potassium, and ammonium adducts are often encountered [5]. These species become possible as the surface excess charge of ESI nanodroplets may be carried by various cationic species [6]. Among these, protons are by far most common; nevertheless, sodium, ammonium, and potassium

cations cannot be neglected. These ions arise from the mobile phase additives, solvent impurities, glassware, and so on.

The ions observed in mass spectra are controlled by several equilibria taking place in the charged nanodroplets produced in ESI. The processes occurring in the ESI droplets, resulting in the observation of these ions in ESI/MS spectra, can be summarized with the following three equilibria that combine two steps.

$$M \overset{H^+}{\rightleftharpoons} [M + H]_l^+ \rightarrow [M + H]_g^+ \tag{1}$$

$$M \overset{Na^+}{\rightleftharpoons} [M + Na]_l^+ \rightarrow [M + Na]_g^+ \tag{2}$$

$$M \overset{NH_4^+}{\rightleftharpoons} [M + NH_4]_l^+ \rightarrow [M + NH_4]_g^+ \tag{3}$$

First, equilibria taking place in the liquid phase (denoted with subscript l) are responsible for the charging of the analyte. Second, after this, formation adducts can be ejected to the gas phase (denoted with subscript g). Although two consequent processes can be distinguished relating to the observation of the analyte signal in the mass spectrum, for the sake of readability, we define the sum of these two processes as the formation of adducts or the formation of protonated molecules, respectively. This definition is further supported by the fact that these two steps are both required to observe a specific species in the mass

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spectrum. It is expected that Equilibria 1–3 coexist (and several other possible ones) as several types of ions can be observed simultaneously in the mass spectrum. However, for some compounds, certain equilibria can prevail and one dominant ion is observed in the mass spectrum.

The observation of one dominant ion in the mass spectrum is often desired. The formation of multiple adducts, together with protonated molecules, is claimed to reduce the sensitivity of each ion [1, 7]. Also, Li et al. [8] observed significant problems with repeatability if only one of the formed ions is monitored. The repeatability could be improved by simultaneously monitoring all formed adducts and protonated molecules, although this approach is tedious as all possible species need to be identified.

Several research groups have found that the formation of adducts can be modified with mobile phase additives. Ma and Kim [9] observed the suppression of sodium adduct formation in analyses of steroids by adding acidic modifier (1% of acetic acid in water phase) to a water/acetonitrile mixture. A contradictory result was observed by Guan et al. [10] for compounds with a similar structure. They observed intensive $[M + Na]^+$ and $[M + K]^+$ adducts, in addition to $[M + H]^+$, in ESI/MS spectra for a mobile phase containing 0.1% formic acid. However, if ammonium formate was used as an additive, $[M + H]^+$ became the predominant ion in the spectra. Hua and Jenke [11] studied the effect of mobile phase pH on the efficiency of sodium adducts formation for hexadecanamide and octadecanamide, and observed an increase the sodium adduct signal with decreasing pH for both amides.

One specific type of additives used to decrease multiple ion formation are alkylamines [7, 12, 13]. In this case, $[M + RNH_3]^+$ ions have been observed as the dominating species. Stefansson et al. [13] and Zhao et al. [7] studied different amines as additives and observed the best sensitivity for primary amines, namely, for dodecylamine (with the longest alkyl chain from the studied primary amines) and methylamine (longer chain amines were not included in the study), respectively. Teshima et al. [12] studied primary alkylamines with different chain lengths and observed increasing sensitivity for $[M + RNH_3]^+$ adducts with increasing chain length up to six carbons. A further increase in chain length was found to decrease the sensitivity. It was also observed that the concentration of the alkylamine plays an important role in the formed adduct intensity [12]. It is obvious that the findings from these different research groups vary significantly.

To reveal the processes behind adduct formation, the various properties of adducts have been correlated with the physicochemical parameters. It has been observed [14] that the solution phase binding constants for adducts do not follow the binding constants observed in ESI/MS. The binding constants in ESI/MS have been found to be on an average two orders of magnitude higher than the ones observed in the solution phase. Also Breitbach et al. [14] did not observe correlation between the limit of detection of adducts and the binding constants, nor between the limit of detection and surface tension decrease, due to the adduct forming agent. However, the solution phase binding was found to be necessary but not sufficient for the observation of adducts; the surface activity was found to have

the dominant effect. We have previously studied [15] the sodium adduct formation efficiency for esters, amines, ketones, and acids, and correlated the results with the hydrophobicity of the formed complex (log$P$) with a bond length between the heteroatom closest to sodium and with the partial charges on the heteroatom bound to sodium. However, none of these parameters alone were able to describe the sodium adduct formation efficiency. Ghosh and Jones [3] associated the ESI/MS response of formate adducts with the nonpolar surface area of the structurally very similar acylsucroses. In general, good correlation was observed, but small variations in sensitivity could not be explained. Therefore, the adduct formation process is widely used and is of significant interest, although the actual mechanism behind it is poorly understood.

One reason for this ambiguity is that different studies incorporate only a small number of compounds and rarely focus on more than two mobile phases. The aim of this study is to assess both of these factors and study how the sodium adduct formation efficiency in ESI positive mode depends on the mobile phase composition. We consider 17 compounds that form both sodium adducts $[M + Na]^+$ and protonated molecules $[M + H]^+$, and have different types of adduct formation sites (four different types in total [15]). The selection of mobile phases covers a pH (water phase) range from to 2.1 to 10.7 and includes all common mobile phase additives used in ESI/MS. Altogether, eight mobile phases are studied. The findings allow us to clarify the relation between observations of various species in mass spectra.

Materials and Methods

Chemicals

Glyceryl triacetate, ethylene glycol diacetate, glyceryl tributyrate, m-methoxybenzoic acid, and 2-cyanophenol were obtained from Sigma Aldrich. Dimethyl phthalate, dimethylmaleate, dimethyl succinate, dimethyl glutarate, and dimethylmalonate were obtained from Merck. Vamidothion and thiamethoxam were from Dr. Ehrenstorfer GmbH. Benzophenone, benzamide, phenyl benzoate, and 1,10-phenantroline were purchased from Reakhim. 2-[(trifluoromethane)sulfonyl]benzoic acid was a kind gift from Professor L. M. Yagupolskii of the Institute of Organic Synthesis, Kiev. As a reference compound, tetraethylamine perchlorate from Fluka was used. The numbers corresponding to compounds can be found in Table 1.

The mobile phase contained 20% ultrapure water (purified with a Millipore Advantage A10 Millipore GmbH, Molsheim, France) and 80% (v/v) acetonitrile (J. T. Baker, Deventer, The Netherlands). As water phase additives, (**A**) 5 mM oxalic acid (Sigma, Saint Louis, USA), (**B**) 0.1% trifluoroacetic acid (Sigma-Aldrich, Saint Louis, USA, LC/MS grade), (**C**) 0.1% formic acid (Sigma-Aldrich, Saint Louis, USA, ≥98%), (**D**) 0.1% acetic acid (Fluka, Reinach, Switzerland, LC/MS grade), (**F**) 5 mM ammonium acetate (Fluka, Buchs, Germany, LC/MS grade), (**G**) 0.1% ammonia (25% solution; Lachner, Brno, Czech Republic), and (**H**) 1 mM methylamine (Sigma-Aldrich, Saint Louis, USA, 40 wt.% solution in water) were

Table 1. Obtained Ionization Efficiency Values for Sodium Adducts (log*SAFE*), Protonated Ions (log*IE*), Ammonium Adducts (log*AAFE*), and Methylammonium Adducts (log*MAFE*). NA Refers to Analyte-Mobile Phase Combinations Where the Ionization Efficiency was Too Low for Accurate Measurements. Water Phase Additives were: (**A**) 5 mM Oxalic Acid, (**B**) 0.1% (8.8 mM) Trifluoroacetic Acid, (**C**) 0.1% (21.7 mM) Formic Acid, (**D**) 0.1% (16.7 mM) Acetic Acid, (**E**) No Additive, (**F**) 5 mM Ammonium Acetate, (**G**) 0.1% (58.7 mM) Ammonia, and (**H**) 1 mM Methylamine

No.	Mobile phase	logSAFE								logIE								logAAFE		logMAFE
		A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H	G	H	H
1	Vamidothion	2.4[a]	4.1[b]	4.5[b]	4.5[b]	5.2[b]	4.0[b]	3.4 a	2.7[a]	3.6	3.1	3.4	2.8	3.9	3.5	3.5	3.9	0.2	NA	2.4
2	Glyceryl tributyrate	3.3[b]	2.9	3.7[b]	4.0[b]	3.9[b]	2.8	2.5	2.1[a]	2.5	2.0	2.4	2.2	3.1[c]	2.9[c]	2.8[c]	0.9	3.0	3.0	3.6
3	1,10-Phenanthroline	1.1[a]	3.0[b]	3.4[b]	3.3[b]	4.2[b]	3.1[b]	2.3 a	1.9[a]	3.0	2.9	3.2	3.2	4.1	3.2	3.0	3.6	NA	NA	NA
4	Glyceryl triacetate	3.0[b]	1.7[a]	3.5[b]	3.6[b]	3.7[b]	1.1[a]	1.9 a	0.6[a]	1.7	1.2	1.6	1.4	2.4[c]	2.1[c]	2.2[c]	0.2	1.8	2.1	2.1
5	Dimethyl phthalate	2.7[b]	2.0[a]	3.2[b]	3.5[b]	4.0[b]	1.4[a]	1.5 a	NA	2.3	1.6	2.2	2.0	2.5	2.7	2.2	2.1	NA	NA	1.1
6	Thiamethoxam	1.6[a]	1.6[a]	3.3[b]	3.6[b]	4.0[b]	2.4[a]	2.2 a	2.0[a]	2.3	1.8	1.6[c]	1.9	2.5	2.2	2.5	3.3	NA	NA	2.7
7	Dimethylglutarat	2.1	1.9	2.8[b]	3.0[b]	3.3[b]	0.8[a]	1.1 a	0.5[a]	1.8	2.0	2.3	1.7	2.6	2.4	2.0	1.2	0.6	0.8	2.0
8	Dimethylmaleate	1.7	1.8	2.5[b]	2.7[b]	2.9[b]	0.6[a]	0.5 a	0.3[a]	1.9	1.8	1.9	1.7	2.2	2.1	1.6	0.9	0.0	0.9	1.2
9	Dimethylsuccinate	1.7	1.7	2.5[b]	2.6[b]	3.0[b]	0.5[a]	0.6 a	0.2[a]	2.0	1.9	2.2	1.7	2.6[c]	2.3	NA	0.6	-0.1	0.1	1.5
10	Phenyl benzoate	1.7[b]	0.6[a]	2.2[b]	2.6[b]	2.7[b]	-0.2[a]	-0.4 a	NA	1.1	1.0	1.5	1.3	1.7	1.6	NA	NA	NA	NA	0.4
11	Ethylene glycol diacetate	1.6	1.5	2.2[b]	2.1[b]	2.4[b]	0.5[a]	0.2 a	NA	1.4	1.4	1.3	1.5	1.3	0.8	0.9	-0.1	0.9	1.4	1.1
12	Dimethylmalonate	1.0	1.0	1.8[b]	1.6[b]	2.1[b]	-0.1[a]	-0.3 a	0.3[a]	1.3	1.2	1.4	1.4	1.5[c]	1.2	0.8	-0.2	0.1	0.2	0.5
13	Benzophenone	1.5	0.7[a]	2.0[b]	2.2[b]	2.4[b]	0.6[a]	0.2 a	-0.6[a]	2.4[c]	1.6	1.7	2.2[c]	2.1[c]	1.6	1.3	0.2	0.5	0.4	-0.5
14	2-(Trifluoromethane)sulfonylbenzoic acid	2.4	1.6	2.2	2.8[b]	2.5	1.1	1.5	1.5	0.6	0.1	0.1	0.4	NA	NA	NA	NA	0.3	0.2	0.0
15	Benzamide	0.7[a]	1.2	1.8	1.6	2.5[b]	1.1	0.7 a	0.8[a]	2.3	2.1	2.1	2.4	2.0	1.8	1.5	1.2	NA	NA	0.6
16	p-Methoxybenzoic acid	1.2	0.6	1.0	1.4	1.6	0.2	0.3	0.4	2.0[c]	1.8[c]	1.3	1.9[c]	0.8	0.5	0.8	-0.1	-0.8	-0.6	NA
17	2-Cyanophenol	0.7	0.5	1.2	1.1	1.8[b]	0.2	-0.4 a	NA	1.6	1.5	1.3	1.7	0.6	NA	NA	NA	-0.1	-0.1	NA

[a] Sodium adduct formation efficiencies that are statistically significantly lower than the ones observed in mobile phase **E** (water/acetonitrile 20/80), giving on average the highest log*SAFE* values

[b] Sodium adduct formation efficiencies that are statistically significantly higher than the ones observed in mobile phase **G** (5 mM ammonia/acetonitrile 20/80), giving on average the lowest log*SAFE* values

[c] Protonated molecule formation efficiencies that are statistically significantly higher than the ones observed in mobile phase **H** (1 mM methylamine/acetonitrile 20/80), giving on average the lowest log*IE* values

tested (concentrations are given relative to the water phase). Mobile phase (**E**) did not contain any additives (water/acetonitrile 20/80 v/v mixture). This mobile phase denotation is used in the figures.

Instrumentation

The measurements were carried out using an Agilent XCT ion trap mass spectrometer (Agilent Technologies, Santa Clara, USA) in ESI positive mode. The MS and ESI parameters were not optimized (except for setting the target masses, see [16]) and the factory defaults of a nebulizer gas pressure of 15 psi, a drying gas flow rate of 7 L/min, and a drying gas temperature of 300 °C were used. The capillary voltage between the MS and nebulizer was –3500 V. All other ion transportation parameters were determined by the *Target Mass* parameter. The ion trap parameters were a smart target (parameter characterizing the number of ions accumulated in the trap) of 100,000 and a maximum accumulation time of 300 ms. Each spectrum was scanned from 30 to 1000 *m/z*. The ICP-MS measurements were performed by the Institute of Geology in the University of Tartu.

Calculations

The formation of protonated molecules (also previously termed ionization efficiency, log*IE*), sodium adduct formation efficiency (log*SAFE*), ammonium adduct formation efficiency

(log*AAFE*), and methylammonium adduct formation efficiency (log*MAFE*) were calculated relative to the tetraethylammonium signal:

$$\log SAFE_M = \log \left[\frac{R([M+Na]^+)}{C_M} \Big/ \frac{R([Et_4N]^+)}{C_{Et_4N^+}} \right] \quad (4)$$

$$= \log \left[\frac{slope([M+Na]^+)}{slope([Et_4N]^+)} \right]$$

$$\log IE_M = \log \left[\frac{R([M+H]^+ + [fragments]^+)}{C_M} \Big/ \frac{R([Et_4N]^+)}{C_{Et_4N^+}} \right] \quad (5)$$

$$= \log \left[\frac{slope([M+H]^+) + slope([fragments]^+)}{slope([Et_4N]^+)} \right]$$

$$\log AAFE_M = \log \left[\frac{R([M+NH_4]^+)}{C_M} \Big/ \frac{R([Et_4N]^+)}{C_{Et_4N^+}} \right] \quad (6)$$

$$= \log \left[\frac{slope([M+NH_4]^+)}{slope([Et_4N]^+)} \right]$$

$$\log MAFE_M = \log\left[\frac{R\left([M + MeNH_3]^+\right)}{C_M} \Big/ \frac{R\left([Et_4N]^+\right)}{C_{Et_4N^+}}\right] \quad (7)$$

$$= \log\left[\frac{slope\left([M_1 + MeNH_3]^+\right)}{slope\left([Et_4N]^+\right)}\right]$$

where R is the response of the compound in ESI/MS and the slope of the analyte signal versus concentration is estimated via linear regression in the linear range of the signal-concentration plot. Also, the isotope distribution of all observed species was taken into account. Previously, it has been observed that for these compounds, only $[M + H]^+$ undergoes fragmentation [15]; therefore, fragments observed in mass spectra are taken into account for logIE calculations. In order to make the data easier to present and analyze, a logarithmic scale (e.g., logIE) has been used. To achieve comparable values of logIE, log-$SAFE$, and so on, all values were anchored to the previously established logIE value of tetraethylammonium of 3.95 [17]. It has also been shown earlier that the change in logIE of tetraethylammonium is not statistically significant between mobile phases with different additives [16]. The analyte concentrations in the linear range were used, depending on the compound and solvent ranging from 10^{-10} to 10^{-7} M.

Computations

The COSMO-RS (COnductor-like Screening MOdel for Realistic Solvents) [18] method was used for generating sigma profiles needed for calculating the weighted average positive sigma ($WAPS$) parameter. COSMO-RS was chosen because it enables calculations in solvent mixtures and is able to handle preferential solvation of solutes by one of the solvent mixture components.

The Turbomole ver. 6.4 software package [19] was used for geometry optimization at the DFT-bp-TZVP level of theory, applying the COSMO continuum solvation model generating input (optimized geometry with sigma surface) modeling of real solvation. COSMOtherm software package ver. C3.0, release 13.0122 was used for the statistical thermodynamics in COSMO-RS. In the input, the solvent composition was given the same as in the experiment (i.e., including the concentration of water in the solvent). Zero concentration (infinite dilution) was used for the studied species. Calculations were performed at 298 K and statistical weighing of conformers was completed by the COSMOtherm program.

$WAPS$ is a parameter for quantitatively evaluating the charge delocalization in anions and is calculated as follows:

$$WAPS = \frac{\displaystyle\int_{\sigma=0}^{\infty} \sigma \cdot p(\sigma)\,d\sigma}{A \displaystyle\int_{\sigma=0}^{\infty} p(\sigma)\,d\sigma} \quad (8)$$

where σ is the polarization charge density on ion surfaces, $p(\sigma)$ is the probability function of σ, and A is the surface area of the anion [20]. The smaller the $WAPS$ value, the more delocalized the charge in the anion. It has been proposed that $WAPS$ values above five indicate anions with localized charge.

The non-polar surface area (NPSA) was calculated using Spartan 08 program package ver. 1.2.0 [21] by first calculating the total surface area and polar surface area of the lowest energy conformer in the mobile phase (evaluated with the COSMO-RS method) and then subtracting the latter from the former.

For comparing log$SAFE$, logIE, and log$AAFE$ values in different media, a t-test with 95% reliability was used.

Results

For the studied 17 compounds, two to four different ions were observed in mass spectra depending on the mobile phases. Protonated molecules and sodium adducts were observed for all compounds; ammonium adducts for 12, and methylammonium adducts for 14 compounds. Also, different types of sodium adducts were observed, mostly $[M + Na]^+$, but for some compounds, $[M_2 + Na]^+$ and $[M - H + 2Na]^+$ were also identified. The dimers $[M_2 + Na]^+$ were taken into account for log$SAFE$ calculations (the intensities for monomers and dimers were summed). $[M - H + 2Na]^+$ was observed only for para-methoxy benzoic acid. However, these ions were not taken into account in the log$SAFE$ calculations, as the link between these species and other sodium adducts is unclear. Ammonium adducts were only observed for mobile phases that contained ammonium acetate or ammonia as an additive (**F** and **G**). Methylammonium adducts were observed only in the mobile phase where methylamine was used as an additive (**H**). The formation efficiencies for all species are presented in Table 1. The simultaneous change of logIE and log$SAFE$ is shown in the example of vamidothion in Figure 1. The examples of obtained mass spectra are shown in Table S1 in the Supplementary Information.

Formation efficiencies, both logIE and log$SAFE$, are linked to the general ionization efficiencies scale [17], where methylbenzoate has been arbitrarily taken as the compound with logIE value of zero. Therefore these values should not be interpreted on the absolute scale, but on the relative scale. This means that at the same concentration, the compound with logIE value of 3 has 10 times higher signal in ESI/MS spectra for $[M + H]^+$ than the compound with logIE value of 2. Assuming that concentrations of both compounds are in the linear range, as logIE and log$SAFE$ values were obtained with a mutual link, it also means that sensitivities for protonated molecules and sodium adducts can be compared directly based on the formation efficiency. For example, a compound that has logIE value 2 units higher than log$SAFE$ value has $[M + H]^+$ peak 100 times more intensive than $[M + Na]^+$ peak.

For each mobile phase, measurements were carried out on at least 3 days. The repeatability standard deviation calculated over all compounds and all mobile phases was 0.30 logarithmic
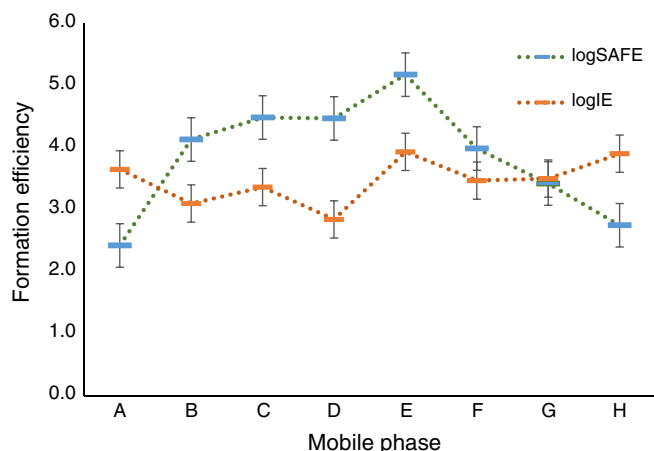
Figure 1. The change of formation efficiencies of protonated molecules (log*IE*) and sodium adducts (log*SAFE*) with mobile phase on the example of vamidothion. The mobile phases are arranged in the order of acidity of the additive. Error bars indicate repeatability standard deviation

units for log*IE* measurements and 0.35 logarithmic units for log*SAFE* measurements, which is comparable to the log*IE* and log*SAFE* scales previously established [15, 17]. However, for most of the mobile phases, the repeatability was significantly better. The worst repeatability was observed for 20/80 water/acetonitrile: 0.67 and 0.50 logarithmic units for log*IE* and log*SAFE*, respectively. An F-test showed that repeatability in mobile phases with additives was statistically significantly better than in mobile phase without additives for log*IE* measurements. The repeatability standard deviations for mobile phases with additives were 0.20 and 0.32 logarithmic units for log*IE* and log*SAFE*, respectively.

The log*SAFE* values varied from 5.2 to –2.3, and significant variation due to additives was observed. In general, the highest ionization efficiencies for sodium adducts were observed in the 20/80 mixture of water and acetonitrile without any additives (mobile phase **E** in Table 1). The statistically significant differences in the log*SAFE* values compared with mobile phase **E** are presented in Table 1 with superscript a. On average, the lowest log*SAFE* values were observed for the 20/80 0.1% ammonia/acetonitrile mixture (mobile phase **G**, statistically significant differences between mobile phase **G** and other mobile phases are marked with superscript b in Table 1). In some cases, the variation of log*SAFE* values between different mobile phases exceeded 3 logarithmic units. For example, for glyceryl triacetate between water/acetonitrile and 1 mM methylamine/acetonitrile the log*SAFE* changed by 3.1 logarithmic units (that is more than 1200-fold increase in the ionization efficiency). Similarly, for vamidothion between the water/acetonitrile mixture and the 5 mM oxalic acid/acetonitrile mixture, the log*SAFE* changed by 2.8 logarithmic units.

The log*IE* values varied to a lesser extent, from 4.1 to –1.5, and were significantly less influenced by the additive than the log*SAFE* values. For all compounds, the variation

in log*IE* values with the mobile phase was up to 2 logarithmic units and did not prove to be statistically significant in most cases. The highest log*IE* values were mostly observed either in 20/80 water/acetonitrile (**E**) or 20/80 1 mM oxalic acid/acetonitrile (**A**) solutions, although statistically significant differences could only be confirmed compared with solvent **H**. The lowest log*IE* values (statistical significant changes in log*IE* are marked with superscript c in Table 1) were observed for the 1 mM methylamine/acetonitrile (**H**) mixture. Statistically significant "wrong-way round ionization" (i.e., an increase in sensitivity/formation efficiencies with increasing pH), was not observed.

Different mobile phase additives also changed the dominant species observed in the mass spectrum. Sodium adducts tend to dominate in weakly acidic mobile phases (mobile phases **C**, **D**, and **E**), whereas protonated molecules dominate in basic phases (**F**, **G**, and **H**). For some compounds, protonated species dominate over sodium adducts also in the strongly acidic mobile phase (**A**). However, it was also observed that the total ion current (the sum of all ion intensities in the mass spectrum) produced by the analyte did vary a lot between different mobile phases. The largest variation of 2.3 logarithmic units (200 times) was observed for phenyl benzoate (between **E** and **H**). This means that although the optimal species for quantitation do depend on the mobile phase additive, the additives also influence the overall efficiency of ion formation.

Log*SAFE* and log*IE* values were in good correlation (R^2 above 0.7) in most of the mobile phases. The best correlation between log*SAFE* and log*IE* was observed for the mobile phase containing ammonium acetate, ammonia, methylamine, or no additives. The R^2 values for the correlation plots were 0.74, 0.74, 0.80, and 0.77, respective to the abovementioned mobile phases. For mobile phase **A**, no correlation between log*SAFE* and log*IE* was observed (R^2 below 0.2).

We also aimed to relate the formation efficiencies with the molecular properties of the formed adduct. Previously, we have shown that the partial charge on the heteroatom, Na-heteroatom bond length (chelating power), and log*P* of the adducts do not correlate with the log*SAFE* values [15]. We also previously studied the molecular properties influencing log*IE*. In both the positive and negative modes, good predicting power for log*IE* has been achieved with charge delocalization and degree of ionization in solution (weighted averaged negative/positive sigma, respectively) [17, 20]. Our previous findings are further supplemented by the work of Ghosh and Jones [3], where a relationship between a compound's response factor and nonpolar surface area was observed. In this study, we tested both the nonpolar surface area (of the neutral form of the analyte) and the weighted average negative sigma (*WANS*, describing the charge delocalization of adducts) in correlation with log*SAFE* (in mobile phase **C**). For the first, a weak positive correlation ($R^2 = 0.56$) was found, whereas for the second, a weak negative ($R^2 = 0.67$) correlation was observed. This means that larger, more hydrophobic compounds with a higher degree of charge delocalization have higher sodium adduct formation efficiencies.

Discussion

Formation Dependence on Mobile Phase Composition

From the results presented in Table 1, it is obvious that mobile phase additives greatly influence the formation efficiencies, and therefore also the spectral features. It is clear that the observed ions and their abundance should be influenced by the concentration of Na^+, NH_4^+, and H^+ in the mobile phase. In this work, the proton concentration is controlled by the additives via pH. The NH_4^+ concentration for solutions **F** and **G**, the only solutions exhibiting ammonium adducts, is also controlled by the mobile phase additives. It is most complicated to control the sodium ion concentration in the mobile phase. Our previous experience has shown that the sodium ion concentration may vary by an order of magnitude between days [15]. The ICP-MS analyses revealed that the sodium concentration coming from the additives used in this study was insignificant compared with the solvent and system sodium concentration. However, our previous study showed, and this was further proven in the present study, that if the measurements are performed in a relative manner, the effect of varying the sodium concentration does not influence the reliability of the obtained results. This is demonstrated by the good agreement of the log*SAFE* scales, which is in the same range as the agreement of log*IE* and log*AAFE* scales (for which the proton and ammonium concentrations are accurately controlled).

Based on the properties of equilibria in solution phase, it can be assumed that Equilibria 1–3 are competitive unless the analyte is present in a large excess, which is usually not the case in ESI droplets. Mobile phase additives can be used to shift these equilibria. Assuming that the only factor affecting the species observed in mass spectra would be competition between Equations (1–3), we would observe a decrease in ion abundance of one species in ESI/MS spectra, while the intensity of another species would increase. Also, the summarized ionization efficiency of all analyte ions (protonated ions plus adducts) should be approximately constant in different mobile phases. For example, when increasing the proton concentration (using acidic additives), one would expect the equilibria to shift towards protonation. The findings of this work are in contradiction to this assumption. First, log*IE* values are less influenced by the additives, although proton concentration in the mobile phase (pH) varied by several orders of magnitude. Second, the highest formation efficiencies for both sodium adducts and protonated molecules were, for most compounds, observed in the same mobile phase (**E**). Also, the total ion current produced by various analyte species was not constant over all mobile phases. This suggests that there are more influential factors affecting formation efficiencies than the competition between the formations of these species in solution.

Interestingly, the log*SAFE* values were dramatically more influenced by the mobile phase additives than the log*IE* values.

This phenomenon could be related to the charge carrier type and properties. It is reasonable to expect that formation of ions detected by mass spectrometry largely occurs on the surface of ESI droplets. This means that sodium adducts are observed because of the surface excess charge carried by sodium ions and protonated molecules due to the part carried by protons (protonated solvent molecules) [6]. Therefore, instead of bulk mobile phase concentrations of the charged species, the droplet surface concentrations are important. Both of these species are expected to be present in reasonable concentrations in all of the studied bulk mobile phases, although proton concentration varies by several orders of magnitude. However, the surface affinities of these species are different. Pegram and Record [22] compared various cations and anions for their accumulation on water–air interfaces. It was observed that protons are strongly surface-accumulated [22, 23], whereas sodium ions are not. Therefore, protons tend to dominate on the droplets surface and the fraction surface excess charge made up by protons is expected to be less influenced by the additives. The more stable log*IE* values observed over various mobile phase additives, in contrast to large variations in the log*SAFE* values, clearly demonstrate that the surface concentration of the charge carrier is of great importance.

Log*SAFE* values for several compounds were much higher than log*IE* values. This reveals that although surface charge is largely expected to be made up of protons, these species have a stronger affinity towards sodium ions compared with protons. As most of these compounds are oxygen bases and have low basicity (pK_a values below 0), it is expected that their affinity towards protons is low.

Also, more specific properties of additives may play a significant role. It can be argued that in the case of acidic buffers, the mobile phase additives, having chelating properties, compete with the analyte for the sodium available in the mobile phase. This is especially likely for mobile phases containing oxalic acid as two closely located carboxylic acid groups have been shown to be prone to adduct formation [15]. This is also supported by the significantly lower log*SAFE* values observed in the mobile phase with the oxalic acid additive, compared with other acidic additives, whereas the log*IE* values were not significantly affected.

It can be assumed that the addition of ammonia to the solution adds the third equilibria (Equilibria 3) to the system. This finding was also confirmed by the observation of $[M + NH_4]^+$ in the mass spectrum of most of the compounds in mobile phases **F** and **G**. As ammonium adducts were observed only for mobile phases with ammonia or ammonium acetate additives, it can be concluded that in the used LC/MS system, the residual ammonium ion concentration is very low and, therefore, these adducts are not observed for all mobile phases. For these two mobile phases, the formation efficiencies of sodium adducts and protonated molecules were significantly reduced for most compounds. This could indicate some competitive nature of different ionization mechanisms. However, the reduced signals of sodium adducts and protonated molecules were also observed for compounds in which the ammonium adducts were not detected.

In mobile phases **F**, **G**, and **H**, protonated molecules tended to be more pronounced than sodium adducts. It has been claimed by Hua and Jenke [11] that ammonium adducts may decompose in the gas phase to protonated molecules and ammonium. This effect may increase the signal for protonated molecules relative to sodium adduct, while both log*IE* and log*SAFE* are still suppressed by the formation efficiency shift towards ammonium adduct formation.

Additives Versus No Additives

In this study, a significant decrease in repeatability was observed while using the mobile phase without additives. This phenomenon is possibly related to the excess surface charge nature. If no additives are added to the mobile phase, the surface charge is made up of ions present in the mobile phase as impurities. These impurities may vary day to day depending on the quality of the water, batch of acetonitrile, and so on. As the ions contribute to the excess surface charge change, the processes occurring in the nanodroplets change. Therefore, the use of mobile phase additives should be encouraged. Our results show that, keeping in mind both the formation efficiencies and repeatability, the oxalic acid additive is recommended for protonated molecule measurements, and acetic acid or formic acid additives for sodium adduct monitoring.

Comparison of Formation Efficiency Dependence on Molecular Properties

The formation efficiencies of different adducts are influenced by ion binding equilibria in the solution phase and the efficiency of adducts escaping to the gas phase via the droplet surface. More hydrophobic ions are shown to ionize better in ESI/MS because of the higher surface affinity [17]. Therefore, it is also interesting to compare log*IE*, log*SAFE*, and log*MAFE* values for the same compound. Adducts between analyte and various cations can have significantly different structures, where the proton may be attached to one part of the molecule, while methylammonium to another. In spite of this, it is expected that the methylamine adducts would be more hydrophobic than the corresponding sodium adduct or protonated molecule. Two compounds (glyceryl tributyrate and glyceryl triacetate) had log*MAFE* values statistically significantly higher than log*IE* values in 80/20 1 mM methylamine solution/acetonitrile. The log*MAFE* values did not exceed the log*SAFE* values statistically significantly in 80/20 1 mM methylamine solution/acetonitrile. However, the maximum log*SAFE* values (over all mobile phases) were statistically significantly higher than the log*MAFE* values, and the maximum log*IE* values did not statistically significantly differ from the log*MAFE* values.

It is suspected that methylammonium may also cause ionization suppression as it is more hydrophobic than the other additives used in this study. This may cause methylammonium to have a significant affinity towards the droplet surface, and therefore reducing analyte access to the surface and reducing the chances for the analyte to be ejected to the gas phase. Comparing ionization efficiency values obtained in ammonia solution with those obtained in methylamine solution can be used

to elucidate the situation, as the pH of the water phase for these mobile phases were closest: 10.7 (ammonia) and 7.9 (methylamine). For glyceryl tributyrate and glyceryl triacetate, log*IE* values in methylamine solution were statistically significantly lower than in ammonium solution. These compounds obey strong chelating properties, and they showed high log*MAFE* values. Simultaneously, the log*IE* values for other compounds were not statistically significantly different in these two mobile phases. Also, the slope values for tetraethylammonium were comparable between mobile phases with ammonium and methylamine additives (as well as for other mobile phases). Therefore, it can be assumed that methylamine itself (in low concentrations) does not suppress the signal for $[M + H]^+$ but the formation of competing adducts may cause some suppression.

The importance of hydrophobicity of the species was also investigated while correlating the log*SAFE* values in one solvent with the molecular properties of the compound. It was observed that both nonpolar surface areas and *WANS* give a reasonable correlation with log*SAFE* values. This reveals that formation efficiencies, not only for protonated molecules but also for adducts, are largely driven by compound surface affinities. This effectively demonstrates that formation efficiencies are affected by (1) compound affinities towards specific ionic species, (2) composition of the excess surface charge, and (3) hydrophobicity of the formed species.

Conclusions

It can be concluded that the dynamics of ionization via protonated molecules and sodium adduct formation efficiencies are not completely explainable with competitive bulk phase equilibria. Also, the formation of multiple ions does not decrease the sensitivity for analyses, as claimed earlier. Additives also improve the repeatability; therefore, using a mobile phase with additives is beneficial for compound forming adducts. We have also shown that if ionization via protonated molecules and sodium adducts occurs, the formation efficiencies for both are driven by the hydrophobicity relating to the affinity for the droplet surface.

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