

Negative Ion Electrospray Mass Spectrometry of Nucleotides: Ionization from Water Solution with SF₆ Discharge Suppression

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The total current and selected ion currents from the electrospray ionization (ESI) of 10⁻⁵ M solutions of cocaine hydrochloride and deoxycytidine monophosphate (dCMP) monosodium salt in methanol and water solvents were compared in positive and negative ion modes, respectively, without and with SF₆ gas as a discharge suppressant. The ESI onset voltages (V_{on}), were the same for the positive and negative ion modes. The V_{on} for methanol was much lower than that for water and in agreement with the equation of D. P. H. Smith, who attributes the difference to the higher surface tension of water. The onset of electric discharge (V_{dis}) without SF₆ occurred at lower capillary voltages for the negative relative to the positive ion mode for methanol; but V_{dis} is much higher than V_{on} for methanol, and discharges do not interfere with ESI operation. For water, $V_{on} \approx V_{dis}$ in the absence of SF₆ in the negative ion mode, and ESI operation is impossible without SF₆ discharge suppression. The discharge problem in the positive ion mode is less severe, but SF₆ is still very useful. A dynamic range of 10⁻⁷-10⁻⁵ M was obtained by selected ion monitoring of [dCMP - H]⁻ at 4.5 and 20 μ L/min. flows. Subpicomole detection limits for the nucleotide salt were obtained under these conditions. (*J Am Soc Mass Spectrom* 1993, 4, 289-295)

We are interested in the potential of negative ion electrospray ionization/mass spectrometry (ESI/MS) for the analysis of nucleoside/nucleotide mixtures by capillary electrophoresis/MS and microbore high-performance liquid chromatography (HPLC)/MS.

The negative ion mode (NIM) has been used for ESI/MS of oligonucleotide anions in solution [1-3]. By contrast, ESI/MS analysis of monomeric nucleotides has received little attention. Fenn and co-workers [4, 5] have provided some NIM spectra for these compounds, but most of the reported ESI mass spectra of single nucleotides [2, 5] were obtained in the positive ion mode. Identification of many analytes by HPLC/MS separations of monomeric nucleoside/nucleotide mixtures has been performed by thermospray/MS [6].

The extensive solution ionization of nucleoside monophosphates, diphosphates and triphosphates requires close to 100% aqueous mobile phase to allow their reversed-phase HPLC or ion-exchange chromatographic separation [7]. The ESI/MS of highly aqueous

solutions poses problems. The high electric field necessary for droplet formation at the ESI spray tip for high surface tension liquids (i.e., water) can initiate corona discharge [8].

The sheath flow ESI device of R. D. Smith et al. [1, 9] appears to perform well for mononucleotides and oligonucleotide in the NIM, as does Henion's "ion-spray" [3, 10]; however, the minimum solution organic content for which ESI mass spectra were shown was 5% acetonitrile [10] in the citations from these investigators [1, 3, 9-12]. Chowdhury and Chait [13] demonstrated that stable ESI currents were obtained for pure aqueous solutions with a specially sharpened and electropolished spray tip in air without nebulization assistance. This study showed ESI mass spectra for horse heart cytochrome *c* in aqueous solution containing 0.01-3% acetic acid levels, but all results were for the positive ion mode.

Yamashita and Fenn [14] observed that the onset of corona discharge occurs at significantly lower field strengths for negative versus positive ion ESI/MS operation. They suggested that the introduction of a high electron affinity gas over the spray tip would be helpful in suppressing discharge. The gases reported in this role to date include O₂ [14], N₂ doped with freon [12], and SF₆ [15].

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Ikonomou et al. [15] provided comprehensive data on the effects of SF₆ discharge suppression for positive ion ESI/MS of cocaine/HCl in 99% water/1% methanol, and we present here the results of subsequent work, showing the successful corona discharge suppression by SF₆ for the negative ion ESI/MS of the DNA nucleoside 5'-monophosphate salts from pure aqueous solutions.

Very recently, R. D. Smith and co-workers [16] reported a study of multiply negatively charged polypeptides and proteins resulting from the deprotonation in basic solution of peptides or proteins with multiple acid functions. Although these workers used the sheath flow device [1,9], they found it advantageous to also use SF₆ gas flow with the sheath flow. This indicates that the use of SF₆ for discharge suppression may become widespread in the future, particularly for work with aqueous solutions and in the NIM.

Experimental

The instrument used in this study was a triple quadrupole mass spectrometer (TAGA Model 6000E; Sciex) equipped with an ESI source in the atmospheric pressure plenum chamber [17]. The source [17] and the ESI sprayer assembly [15] have been described in detail elsewhere. Briefly, the analyte solution is electrosprayed at the tip of a [0.1-mm inner diameter (id), 0.23-mm outer diameter (od)] stainless steel capillary. The SF₆ gas is conducted through the annular space between an outer tube (1.6-mm od, 1.3-mm id) and the inner capillary to "blanket" the ESI tip. The capillary protruded 4.4 mm beyond the outer tube. Analyte solution was supplied to the capillary through 0.075-mm id, 0.275-mm od fused-silica tubing joined to the capillary by means of a zero dead volume union. Solutions were supplied by continuous infusion to the sprayer from a motor-driven syringe. The flow rates were 20 μ L/min (unless otherwise indicated), and the solvents were various methanol/water mixtures and the pure liquids. The methanol was reagent grade, redistilled with H₃PO₄ to remove any amine impurities. The water (H₂O) was doubly-deionized by treatment in a Millipore MILLI-Q water purification system ($6 \times 10^{-6} \Omega^{-1} \text{ m}^{-1}$). The solvent compositions are given in volume percent. The SF₆ used was a chemically pure grade from LINDE.

The SF₆ flow used was 200 mL/min [15]. The relatively wide i.d. of the SF₆ sleeve led to low SF₆ gas velocities near the inner capillary tip. This slow flow suppressed corona discharge while avoiding nebulization, thus attaining minimal disturbance of conventional ESI conditions. An ambient air flow of approximately 500 mL/s was maintained through the atmospheric pressure plenum chamber.

The ESI sprayer assembly was mounted inside the plenum chamber. The capillary tip-to-interface plate wall separation distance was 4 cm, and the capillary

was 0.5 cm off-axis from the 4-mm-diameter orifice in the interface plate.

The 4-cm distance between capillary tip and the counterelectrode (interface plate) is larger than distances used in other laboratories. We use a larger distance because it reduces the penetration of unevaporated droplets into the interface chamber. Other interfaces handle that problem in different ways. The larger electrode distance necessitates larger capillary potentials for ESI; however, this does not mean that the present arrangement is more prone to electric discharge. To obtain a given electrospray emission, a given electric field is required. If the required electric field is too high, as in the case for water, then an electric discharge will occur. Although the voltages with different electrode arrangements will differ, the required electric field for ESI at the tip will be the same. Therefore, the results presented in this work also remain relevant for other interfaces that use lower capillary voltages and shorter distances.

The electric fields driving the electrospray and the corona discharge are dependent on the potential difference between the capillary tip and the large counterelectrode (interface plate). Therefore, all the capillary voltages quoted correspond to that potential difference and not to the capillary potential relative to ground. The capillary potential relative to ground is 650 V higher because the interface plate is at 650 V relative to ground.

Results and Discussion

Corona Discharge in Ambient Air and Its Suppression

Because one objective was to examine the extent of discharge suppression by SF₆ in ESI of methanol and water solutions, it was found useful to examine the nature of the discharge-produced ions in ambient air when the capillary and capillary tip are dry (i.e., in a conventional corona discharge originating at the capillary tip).

A relatively steady discharge current of 3×10^{-8} A was obtained at -3.4 kV. The major ions observed were at m/z 32 (O₂⁻), 45 [HCO₂⁻ (formate)], 46 [NO₂⁻ (most intense)], 62 (NO₃⁻), and 89 [CH₃CHOHCO₂⁻ (lactate?)]. These are similar to the background ions observed by Yamashita and Fenn [14] (O₂⁻, NO₂⁻, O₃⁻) and Henion and co-workers [12] [HCO₂⁻, CH₃CO₂⁻ (acetate), NO₃⁻ and CH₃CHOHCO₂⁻ (lactate)] for NIM electrospray, respectively.

An increase in the capillary voltage in the present work to -5.4 kV led to a total current of 1.9×10^{-6} A and the negative ion spectrum shown in Figure 1a. The intensities of all of the ions observed at the lower voltage increased, as shown in Figure 1a. In addition, ions at m/z 16 (O⁻), 17 (OH⁻), 60 (CO₃⁻), 61 (HCO₃⁻) and 77 (HCO₄⁻) became prominent. These species are

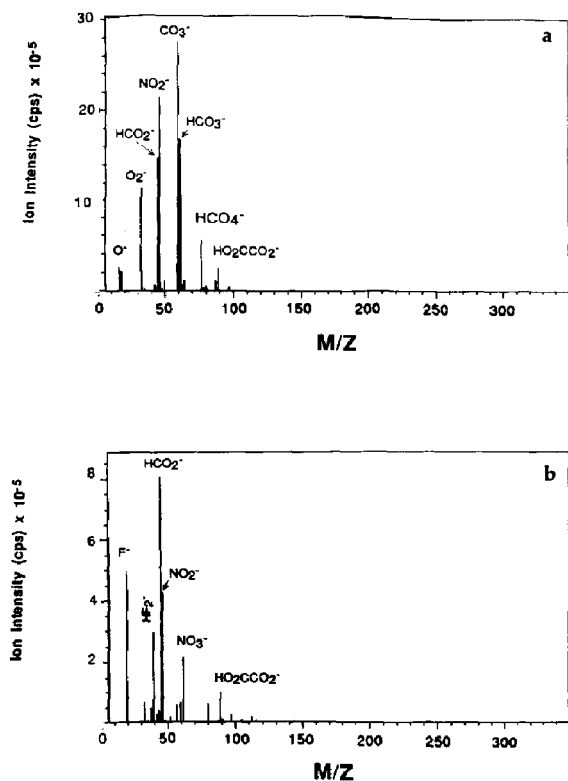


Figure 1. Mass spectra observed in the presence of electric discharge when the capillary tip was dry. (a) Mass spectra were obtained in the absence of SF_6 flow; capillary voltage $V_c = -5.4$ kV; $\text{TC} = 1.9 \times 10^{-6}$ A. Ions observed are typical discharge ions. (b) Mass spectra were obtained with SF_6 flow; $V_c = -5.4$ kV; $\text{TC} = 3 \times 10^{-8}$ A.

all consistent with what is known about negative ion chemistry in the troposphere and ionosphere [18, 19].

In the presence of SF_6 flow at 200 mL/min, the onset of the discharge increased from -3.4 to -5.0 kV. A mass spectrum obtained at -5 kV with SF_6 is shown in Figure 1b. The total current at -5 kV dropped from 1.9×10^{-6} to 3.0×10^{-8} A in the presence of SF_6 , and the total intensity of the ions (Figure 1b) became much lower. The relative ion intensities of O^- , O_2^- , and NO_2^- are considerably reduced in the presence of SF_6 , whereas some new SF_6 -derived ions (F^- and HF_2^-) appeared. The change is in line with the expected interception of electrons by SF_6 , which disrupts the electron "avalanche" [20]. The SF_6^- formed by electron capture [20] is not observed in the spectrum (Figure 2b). Grimsrud and co-workers [21b] showed that SF_6^- in the presence of H_2O is converted to F^- , and it is probably largely this reaction that removes the SF_6^- and leads to the observed F^- .

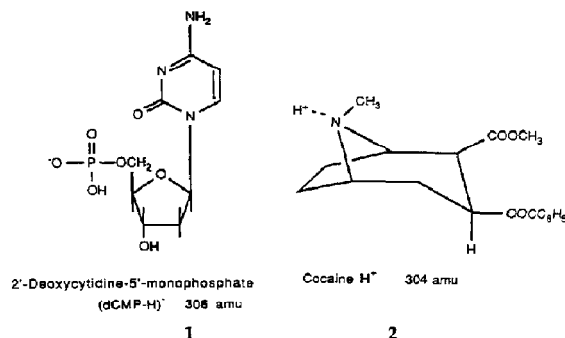
The results above demonstrate that at lower capillary voltages, SF_6 can lead to complete suppression of the discharge. At higher voltages, such as -5.4 kV, a very large reduction in the discharge still occurs, lead-

ing to current decreases by a factor of approximately 60, but a total suppression is not achieved.

The onset of electric discharge in the positive ion mode (PIM) was also determined with a dry capillary. Several tests in the absence of SF_6 showed that the PIM onset occurs at potentials higher by approximately 600 V relative to the onset in the NIM. The onset of electric discharge observed with a dry capillary for NIM and PIM is summarized in Table 1. The nature of positive ions formed by electric discharge in air has been described in previous work [15].

Mass Spectra and ESI Analyte Ion Mass Spectral Detection: Electric Discharge Characteristics

Mass spectra obtained from solutions of 10^{-4} M deoxycytidine monophosphate monosodium salt (NadCMP) in 100% H_2O at -4.4 kV without and with 200 mL/min SF_6 flow are shown in Figure 2. The structure of the $[\text{dCMP} - \text{H}]^-$ anion, formed by the near-100% dissociation of NadCMP in aqueous solution, is shown in Structure 1.



The spectrum without SF_6 (Figure 2a) contains the same ions as were observed from the dry capillary discharge (Figure 1a). Notable is the complete absence of the ESI analyte ion $[\text{dCMP} - \text{H}]^-$. In the presence of SF_6 (Figure 2b), the total intensity of the discharge ions

Table 1. Electrospray and Electric Discharge Onset Voltages for Methanol and Water^a

Solvent	Mode	V_{on} theory (kV)	V_{on} (kV)	V_{dis} (kV)	V_{dis} (SF_6) (kV)
Methanol	NIM	-2.2	-2.1	-3.6	-4.5
	PIM	+2.2	+2.1	+4.6	+6.0
Water	NIM	-4.0	-3.7 ^b	ca. -3.7	ca. -4.5
	PIM	+4.0	+3.8 ^b	ca. +4.0	ca. +5.5
Dry capillary	NIM	—	—	-3.4	-5.0
	PIM	—	—	+4.0	ND ^c

^a V_{on} , onset of electrospray; V_{dis} , onset of electric discharge; potentials quoted are relative to counterelectrode (interface plate), which is at +650 V PIM and -650 V NIM relative to ground.

^b Determined in the presence of SF_6 flow.

^c ND, was not determined.

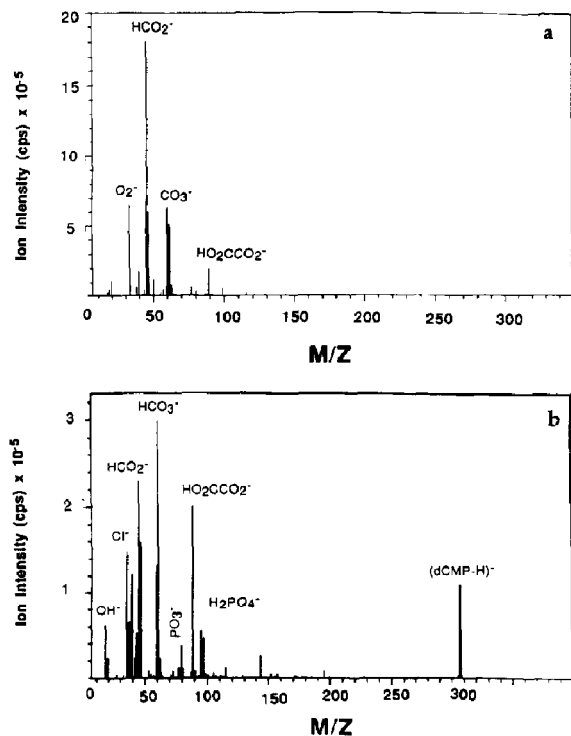


Figure 2. Mass spectra obtained from ESI of 10^{-4} M solutions of NadCMP in 100% water at $V_c = -4.4$ kV. (a) Without SF_6 flow no analyte ion can be detected; (b) with 200 mL/min SF_6 flow, the anion $[\text{dCMP} - \text{H}]^-$ is detected.

has decreased by a factor of approximately 10, and $[\text{dCMP} - \text{H}]^-$ is present with substantial intensity.

The total current (TC) to the counterelectrode (interface plate) and the intensity dependence of selected ions plotted versus the capillary voltage are shown in Figures 3 and 4. The results provide data illustrating the effect of the SF_6 flow on ESI in the NIM with 10^{-5} M NadCMP with methanol and water as solvents. From Figures 3 and 4 one can deduce three capillary voltage (V_c) potentials that are of interest: onset of electrospray (V_{on}); onset of electric discharge in the absence of SF_6 (V_{dis}); and onset of discharge in the presence of SF_6 [$V_{\text{dis}}(\text{SF}_6)$], V_{on} was chosen as the point at which there is a sudden appearance of TC. A sharp onset is also observed in the intensity of analyte ions at a potential that is very close to that indicated by the TC. The average V_{on} values are summarized in Table 1.

Also included in Table 1 for comparison are the corresponding potentials for the PIM mode where cocaine HCl (2) was used as analyte. These data were obtained on the basis of measurements performed in the present work under exactly the same conditions as the NIM experiments. The PIM experiments were a duplication of the earlier work [15], and because the results were very close to those obtained earlier (see

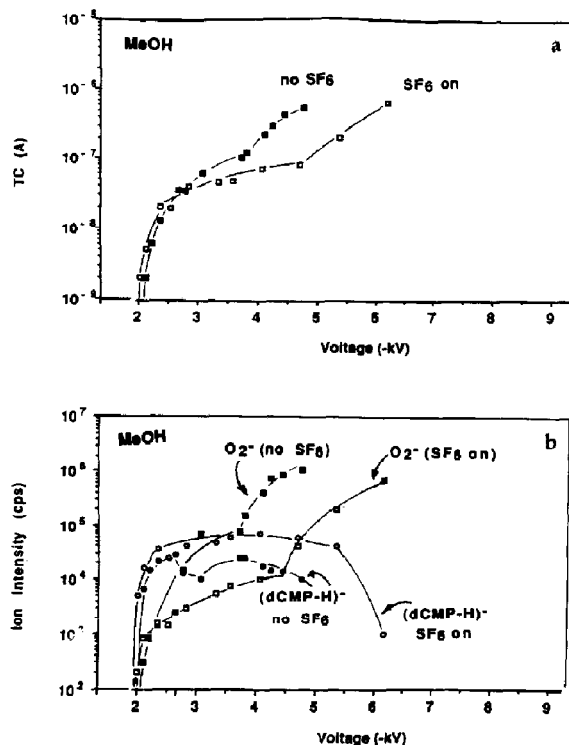


Figure 3. (a) Capillary TC; (b) mass-analyzed ions. Both were observed with 10^{-5} M solutions of NadCMP in methanol (MeOH) in the NIM without and with SF_6 gas and plotted versus capillary voltage used. Upward breaks in TC and discharge ions (O_2^-) indicate onset of electric discharge.

Figures 5 and 6 in ref 15), no additional experimental documentation is provided here.

The V_{on} for NIM and PIM is seen to be identical when the same solvent is used. As discussed previously [15], the much higher V_{on} for water is due to the much higher surface tension of water. The V_{on} can be predicted by eq 1, derived by Smith [8],

$$V_{\text{on}} = \left(\frac{\gamma r_c \cos \theta}{2 \epsilon_0} \right)^{1/2} \ln(4d/r_c) \quad (1)$$

where γ is the surface tension (for H_2O , $\gamma = 0.073$ N m^{-1} ; for CH_3OH , $\gamma = 0.0224$ N m^{-1}); $\theta = 49.3^\circ$ (half-angle of Taylor cone); $\epsilon_0 = 8.8 \times 10^{-12}$ J $^{-1}$ C 2 m $^{-1}$ (permittivity of vacuum); $d = 0.04$ m (distance between capillary tip and counterelectrode), and $r_c = 1.15 \times 10^{-4}$ m (outer radius of capillary tip).

Smith's derivation of eq 1 is not dependent on the sign of the charged droplets formed, which means that the V_{on} should be the same for NIM and PIM when the same solvent is used. The values for V_{on} predicted by eq 1 for the present experimental conditions are shown in Table 1 and are seen to be in good agreement with the experimental results.

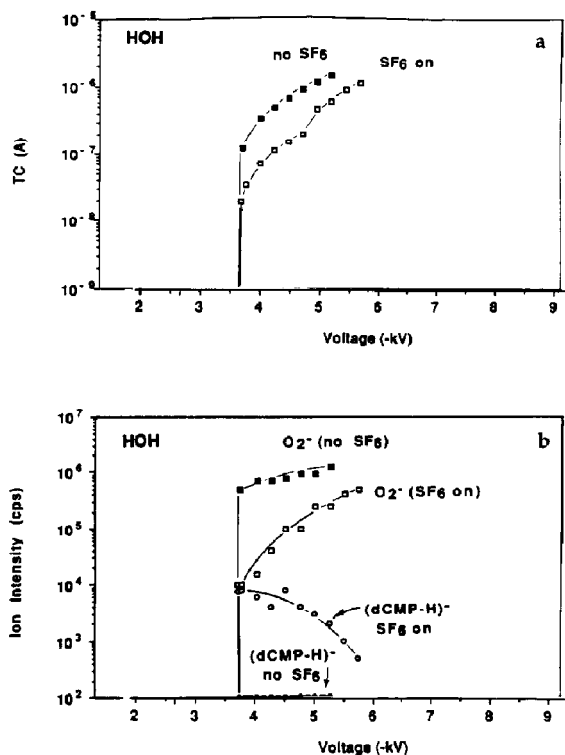


Figure 4. Capillary TC and mass-analyzed ions from 10^{-5} M solutions NadCMP in water (HOH), NIM. ESI onset for water occurs at higher capillary voltage, where an electric discharge also occurs. No analyte ion can be observed without SF₆. The presence of SF₆ allows detection of analyte ion.

As in the previous work [15], we select for V_{dis} the potential at which an upward break is observed in the TC. These breaks are not always distinct. The curves for the discharge-indicating ions provide additional information. The choice of O₂⁻ as the discharge-indicating ion for NIM is justified by the dry discharge mass spectra (see Figure 2a). The discharge-indicating ions for NIM and PIM in methanol [15] show discharge breaks at the same position as the TC break, and therefore the determination of V_{dis} and $V_{\text{dis}}(\text{SF}_6)$ for methanol NIM and PIM is unambiguous (see Figure 3) (see also Figure 5 in ref 22). The V_{dis} values for methanol are given in Table 1.

Because the electrospray onset V_{on} for methanol is low, the electric discharge occurs relatively late, and discharge interference is not a serious problem for PIM or NIM in methanol. Some differences between NIM and PIM should be noted. The discharge occurs later for PIM: $V_{\text{dis}} = 4.6$ kV (PIM); $V_{\text{dis}} = 3.6$ kV (NIM). SF₆ flow delays the discharge by 1.5 kV for PIM and only 1.0 kV for NIM. A later onset of discharge from PIM was also observed with the dry capillary. As was the case for the dry capillary, the earlier occurrence of discharge for NIM is probably due to discharge onset by field emission of electrons from fine tips and

"whiskers" at the capillary tip. This type of charge emission is not possible for PIM.

At higher discharge currents where $\text{TC} > 0.5 \mu\text{A}$, decreases of the analyte ion intensities are observed for NIM (see Figure 3) and PIM (see Figure 5 in ref 15). Because of the earlier onset of discharge for NIM, the analyte intensity decrease occurs earlier for NIM; however, for both PIM and NIM in methanol there is a plateau region in analyte ion intensity at lower voltages, particularly in the presence of SF₆, which allows a comparison of the analyte ion intensities in the absence of discharge. This "best" analyte ion intensity is much higher for the PIM analyte [cocaine + H]⁺, approximately 4×10^5 HZ [15] relative to the NIM [dCMP - H]⁻ ion, approximately 5×10^4 HZ. Mass-dependent quadrupole transmission cannot be responsible for this difference because the two ions have nearly the same mass, m/z 304 and 306. Probably, the intensity disparity is also not due to other NIM versus PIM differences. Most likely involved are the different structures of the ions, (see structures 1 and 2) [dCMP - H]⁻ has more polar, hydrogen-bonding groups than [cocaine + H]⁺. A lower efficiency for the electrospray conversion of ions in solution to ions in the gas phase is expected for the more polar, hydrophilic ions such as [dCMP - H]⁻ [22, 23].

The onset of electrospray in water requires a much higher potential ($V_{\text{on}} \approx 3.8$ kV), which is in the region of discharge onset with a dry capillary ($V_{\text{dis}} \approx 3.4$ kV NIM, 4 kV PIM; Table 1). Therefore the occurrence of discharge close to the electrospray onset, observed in Figure 4 for NIM (and in Figure 6 in ref 15 for PIM) is not surprising. In the absence of SF₆, the discharge interference is far more pronounced for NIM. This is clearly indicated by the much higher discharge-ion intensity (O₂⁻ $\sim 10^6$ HZ) at the onset for NIM relative to CH₃OH₂⁺ intensity of approximately 10^4 HZ at onset for PIM [15]. The analyte ion is completely suppressed by the discharge for NIM, whereas a good analyte ion intensity is observed for PIM [15]. Therefore the application of SF₆ is absolutely essential for NIM, whereas for PIM, the presence of SF₆ is beneficial but not essential.

The TCs and the discharge-ion intensities indicate that SF₆ is more effective in suppressing the discharge for PIM relative to NIM. This effect may also be due to field emission of electrons in NIM. The electrons emitted at the sharp points are exposed to very high electric fields and will accelerate rapidly to high velocities, where electron capture by SF₆ is less efficient.

As noted above, the presence of discharge current reduces the intensities of ESI-produced ions. This is evident for both NIM and PIM in methanol and water (Figures 3 and 4 and ref 15). In our previous work [15], we attributed this adverse effect to the increased space charge at higher currents. The presence of space charge in the vicinity of the interface plate and sampling orifice may be expected to reduce the fraction of ions that enter the mass analysis region. Fenn et al. [24]

suggested another possible cause. The presence of electric discharge reduces the electric field at the capillary tip, and this leads to a reduction in charging of the surface of the liquid tip [24]. One may speculate that this in turn leads to reduced formation of highly charged droplets of very small radius, which are the most efficient sources of gas-phase ions [25].

ESI Mass Spectra of DNA Nucleotides in 100% H₂O Analyte Signal Stability and Dynamic Range

The results in Figures 2b and 4b showed the successful NIM ESI/MS analysis of NadCMP in aqueous solution when SF₆ is used to suppress discharge. Under the same experimental conditions, very similar spectra were also obtained for neat aqueous solutions of sodium 5'-monophosphate of 2'-deoxythymidine (TMP), 2'-deoxyadenosine monophosphate (dAMP), and 2'-deoxyguanosine monophosphate (dGMP). In all cases, the [M - H]⁻ peak was by far the major analyte ion. The dCMP, TMP, and dAMP had very similar [M - H]⁻ ion intensities, 10⁵ HZ for 10⁻⁴ M solutions, whereas dGMP led to an [M - H]⁻ ion of approximately 0.5 × 10⁵ HZ.

The signal stability of the [M - H]⁻ ions obtained with ESI of 10⁻⁴ M aqueous solutions of NadCMP and NadAMP observed in single-ion monitored plots was approximately ±8%. The electric discharge-generated ions (HCO₃⁻, HCO₂⁻, and O₂⁻) were also followed. It was found that the O₂⁻ intensity fluctuated widely by close to a factor of 10 with rise times in the 1-s domain. It was shown in Figure 1 that the O₂⁻ ion intensity was most strongly affected by the presence of SF₆. The large fluctuations therefore should reflect turbulence and uneven mixing of the SF₆ gas into the atmospheric gas near the capillary tip. Increase of the SF₆ flow from 200 to 350 mL/min decreased the O₂⁻ signal by a factor of approximately 2 to approximately 10³ HZ and also its fluctuation. An increase in the [M - H]⁻ signal stability to where the signal deviation was only ±2% was also observed for these conditions. Still better analyte ion signal stabilities were obtained at lower solution flow rates such as 5 μL/min.

The ESI/MS dynamic range in neat water is illustrated in Figure 5, which shows the change in TC and analyte ion [M - H]⁻ intensity with change in concentration of the analyte NadCMP. A linear region in the log - log plot with slope approximately equal to unity is observed for the concentration range 10⁻⁷-10⁻⁵ M. This region in which the ESI analyte ion intensity is proportional to the analyte ion concentration in the solution has been observed in PIM and discussed previously [17,23]. The gradual flattening out of the ion intensity above 10⁻⁵ M solution concentrations (Figure 5b) also follows the typical pattern observed for PIM in previous work [17,23]. The ion intensities shown in Figure 5b demonstrate that higher analyte ion intensities can be obtained at the lower solution flow rates of 4.5 μL/min.

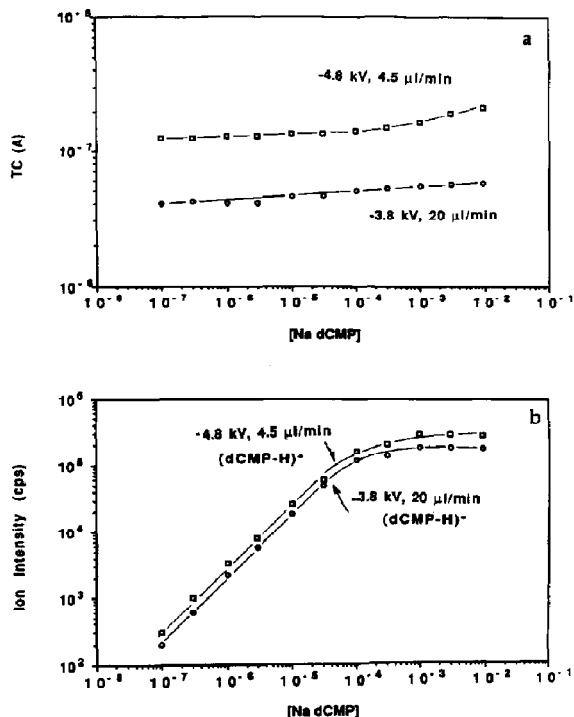


Figure 5. ESI/MS dynamic range for a typical nucleotide (dCMP). Plots of capillary TC and analyte ion [dCMP - H]⁻ intensity versus analyte concentration in moles per liter.

The detection limits for NadCMP can be estimated from data in Figure 5b. At 3 × 10⁻⁷ M analyte concentration, the detected ion intensity is approximately three times the signal deviation. For the approximately 8 s required to collect a mass spectrum, the required amount of analyte in solution at 4.5 μL flow rates is approximately 0.2 pmol. This illustrates that very low detection limits are possible with aqueous solutions of analytes despite the problems created by the high surface tension of water and the enhanced propensity for electric discharge in the NIM.

Conclusions

Water relative to methanol and other solvents used in ESI has a much higher surface tension that requires higher capillary voltages for operation of ESI. This is predicted by theory [8] and observed by experiment in the present and previous work [15] from this laboratory.

The high ESI operating voltages required for water lead to the presence of simultaneous electric discharges that reduce the sensitivity for mass spectrometric detection of ESI analytes. The electric discharge can be suppressed and the analyte sensitivity improved by the application of SF₆ gas.

Comparisons between methanol and water as solvents in the NIM and PIM reveal the following features. The ESI onset voltages are the same for NIM and PIM, as predicted by theory [8]. Discharge onset occurs sooner and suppresses analyte ion detection sensitivity more severely for NIM than PIM. Owing to the low operating ESI voltage required for methanol, application of SF₆ is of no advantage for PIM. Owing to the high operating ESI voltage required for water, electric discharge occurs for both NIM and PIM, and the application of SF₆ is essential in both modes, however, for PIM it is still possible to obtain analyte ion signals without SF₆, whereas for NIM, analyte detection is very difficult without SF₆.

The greater tendency toward electric discharge for NIM relative to PIM is attributed to electron emission for the metal capillary tip for NIM. Although the discharge problems are greatest with water in the NIM mode, the application of SF₆ leads to stable ESI analyte ion signals and detection limits for nucleotide anions in the subpicomole range.

Acknowledgment

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