Kinetics of Solvent Addition on Electrosprayed Ions in an Electrospray Source and in a Quadrupole Ion Trap

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Abstract :

Benzylpyridinium cations readily fragment in the electrospray source by loss of pyridine to give benzyl cations (M - 79). The full-scan spectra obtained with some instruments also show, in addition, an *m/z* (M - 38) peak corresponding to the addition of acetonitrile, being present in the solvent mixture, on the benzyl cations. Here we report that the addition reaction can occur in the source region of ES-MS instruments, and in a quadrupole ion trap. The kinetics of acetonitrile addition was monitored in an ion trap, acetonitrile being provided by leakage from the source, through the heated capillary. For benzyl ions with different substituents, the addition kinetics has been found positively correlated with the Brown parameter σ^+ of the benzyl radical, and therefore with the effective charge density on the α-carbon atom of the benzyl ion. This is consistent with the Langevin or ADO theory of ion-molecule reaction kinetics.

Introduction :

The unimolecular dissociation of benzylpyridinium ions (reaction 1, figure 1) has been already studied by our group for the determination of the internal energy of ions produced by electrospray sources [1-3]. The extent of fragmentation is correlated with the activation energy (or energy barrier E_b) for dissociation. If it is assumed that the dissociation proceeds via a loose transition state (simple bond cleavage) [5,6] with no reverse energy barrier (see figure 2), E_b is assumed to be equal to $\Delta H_f(F^+) + \Delta H_f(Py) - \Delta H_f(M^+)$ [4]. According to the nature and the position of the substituent R, the energy barrier changes: the more electron-withdrawing the substituent, the higher the energy barrier for pyridine loss, and the slower the reaction 1. The electron withdrawing character of the substituent can been quantified in solution by the Hammett σ empirical parameter [7]. In the following we will use the values σ^+ given by Brown et al. [8], as these are better suited for positive charge stabilization [9].

During our investigation of benzylpyridinium fragmentation on different electrospray sources, we were faced with a new "fragment", which turned out to be an acetonitrile adduct on the benzyl fragment. Acetonitrile was used in the solvent mixture. The present article describes these results, and the subsequent study of the addition kinetics in a quadrupole ion trap mass spectrometer.

Experimental :

The first instrument used in this study is a Zabspec-T sector mass spectrometer (Micromass, Manchester, UK). The first mass analyzer (E_1BE_2) was used in all experiments. The mass spectra were acquired using an alpha station under the control of an OPUS 3.1 data system. Ion detection was achieved by using a photon multiplier located after the second electrostatic analyzer (E_2). Electrospray ionization was performed with an atmospheric pressure ion source fitted with a hexapolar ion guide. The samples were dissolved in water/acetonitrile (1/1–v/v) at a concentration of 10^{-4} M. The solution was infused continuously into the ion source at a flow rate of 5 μL min^{-1} by using a syringe pump (Harvard Apparatus, Model 11). The electrosprayed ions were introduced into the mass analyzer at 4 keV kinetic energy. For the in-source CID experiments on the Zabspec-T instrument, the sampling cone voltage was increased by 4.8 V steps from 4000 to 4096 V. The skimmer voltage was kept constant at 4000 V. Mass spectra were obtained by B scans of 5 s per decade in the range 550-50 Th. Resolution was 1000 at 10 % valley. The ion peak intensity values were calculated by averaging the signals measured on each set of three scans.

The second instrument used is a Finnigan LCQ (ThermoQuest, Bremen, Germany), operated in the positive ion mode with a needle voltage of 4.7 kV and a tube lens offset of –50 V. The benzylpyridinium ion concentration was 5.10^{-6} M in water/acetonitrile (1/1–v/v). For the study of in-source fragmentation, the capillary voltage was increased (with the skimmer at ground) at fixed capillary temperature (140 °C). The flow rate was 4 µl min^{-1}. The full-scan mass spectra were acquired for 50 scans. In standard operation mode, the mass range was kept between 50 and

250 Th. For the study of acetonitrile addition reaction in the trap, the benzylpyridinium (M^+) ion was selected in MS^2 and activated at 50% amplitude during 2 ms. The helium buffer gas pressure (10^{-3} torr) and the activation q_z (0.25) were kept constant. The corresponding benzyl fragment (M-79) was selected in MS^3 and allowed to remain trapped for different time intervals with no further activation (activation amplitude of 0% and activation time varied from 10 ms to 10 s). Unless specified, a flow rate of 4 µl/min, a capillary temperature of 140 °C, a capillary voltage of 5 V and a tube lens offset of –50 V were used for all the MS^n experiments.

Results:

The fragmentation of benzylpyridinium cations (M^+) has been studied by source-CID on the ZabSpec-T. The fragmentation involves the loss of neutral pyridine producing a fragment F^+ at mass M - 79 (see figure 1). Figure 3 shows the full-scan mass spectra of m-OCH_3, m-CH_3, p-CH_3, and p-OCH_3-substituted benzylpyridinium cations. Surprisingly, another fragment was also found at M - 38. As the carrier solvent was water/acetonitrile (1/1), this "fragment" was attributed to the addition of acetonitrile on the benzyl cation F^+ (M - 38 = M - 79 + 41, reaction 2). In the text below, we will use the following notations (see figure 1): M^+ for the benzylpyridinium ion, F^+ for the benzyl fragment and Add^+ for the acetonitrile adduct on F^+. For each substituent, while the relative abundance of F^+ increases as the sampling cone voltage increases, the ratio between Add^+ and F^+ remains constant (figure 4). Table 1 lists the different substituents of the studied compounds, ranking from the most to the less prone one to acetonitrile addition. The table also summarizes the different results obtained throughout this study, as well as the Brown σ^+ parameters [8], available for *meta* and *para* substituents.

Apparently, such acetonitrile adducts cannot be observed on all electrospray mass spectrometers: source-CID of benzylpyridinium in water/acetonitrile (1/1) ions were already performed on a VG Platform and on a PE Sciex API 165 [2], but no such adducts have ever been observed. On a Bruker Esquire ion trap instrument, small amounts of acetonitrile adducts were observed, only for compound **1** (T. Gougnard, personal communication). On the LCQ instrument, however, adducts could be detected in full scan MS spectra of compounds **1-8**. The ratio of the intensities $I(Add^+)/I(F^+)$ are summarized in Table 1.

If adducts are formed by recombination with acetonitrile, one could observe their formation in the ion trap, provided that the acetonitrile partial pressure is sufficiently higher or that sufficiently long trapping times can be achieved. The kinetics of the addition reaction could be monitored for compounds **1-6** by performing MS^3 experiments: M^+ was selected and activated in MS^2 during 2 ms (almost no adduct was formed during this short time), then the fragment F^+ was selected for MS^3 and allowed to remain trapped for different periods (see experimental section for details). The kinetics of addition of acetonitrile on F^+ ions was monitored within that time window. We checked that the addition rate constant did not depend on the extent of activation of the parent ion (data not shown). Figure 5 shows the variation of the intensities of F^+ and Add^+ for compounds **1-3** as a function of the reaction time in the trap. After 10 s (the maximum time allowed by the instrument), a stationary state is attained for compound **1**, and almost attained for compounds **2** and **3**.

Initial reaction kinetics were studied for compounds **1-6**, assuming that the ratio of the intensities in the spectra was proportional to the ratio of the concentrations in the trap after the given reaction time. The plot of $-\ln(I(F^+)/I(F^+)_0)$ as a function of time is linear (Figure 6) only at the beginning of the reaction ($-\ln(I(F^+)/I(F^+)_0) < 2$), before the reverse reaction starts to take place to a significant extent. The rate constants $k_{exp} = -d\ln(I(F^+)/I(F^+)_0)/dt$ observed for the different substituents are summarized in table 1. For each spectrum, $I(F^+)_0$ is taken as the sum of $I(Add^+)$ and $I(F^+)$. As acetonitrile is the solvent, its partial pressure could be varied simply by varying the infusion flow rate. The kinetics of acetonitrile adduction on (m-CN)F^+ was monitored at fixed capillary temperature (140 °C), varying the flow rate from 2 to 8 µl/min (Figure 7). Figure 7 shows that k_{exp} depends linearly on the flow rate. We also investigated the influence of the source heated capillary temperature on the addition kinetics (data not shown), and found that the higher the source capillary temperature, the slower the addition kinetics; the dependence is not linear.

Discussion :

1. Kinetics of CH_3CN addition monitored in the trap

The addition reaction 2 is first order in F^+ (figure 6), and can be considered as first order in acetonitrile: as the dependence of k_{exp} on the flow rate is linear, it can reasonably be supposed that the concentration of acetonitrile in the trap depends linearly on the flow rate, and that k_{exp} depends linearly on [CH_3CN]. We therefore have $k_{exp} = k \cdot [CH_3CN]$, the values of which are listed in table 1. Varying the solvent flow rate is a very easy way of varying the solvent partial pressure in the trap. The capillary temperature also influences the partial pressure in the trap: increasing the capillary temperature decreases the gas density, but it should be stressed that even at 300 °C and with moderate flow rates, the presence of solvent vapor eventually in the trap is non-negligible. As all the rate measurements in figures 5-6 were performed with the same solvent flow rate and the same capillary temperature, the acetonitrile partial pressure in the trap is the same in all the measurements, and the ratios of the k_{exp} values are equal to the ratios of the bimolecular rate constants k. We see in table 1 that k_{exp} varies according to the substituent, the addition reaction being faster for more electron withdrawing substituents. Figure 8 shows the correlation between ($k \cdot [CH_3CN]$) and the Brown σ^+ parameters [8]. The correlation is not perfect, and this can be explained by the fact that σ^+ parameters have been tabulated for typical reactions occurring in solution [9]. For example, an inversion between m-CN and p-CN benzyl ions has also been found for the gas-phase ionization potential of the corresponding benzyl radicals [10]. We also see that the position of the substituent is important for the addition reaction kinetics; this shows that F^+ is the benzyl cation, and not the tropylium ion isomer.

Whether for the dissociation reaction the effect of the substituent is easily explained by the variation of the activation barrier E_b, similar arguments cannot be used for the association reaction, as we assume that there is no reverse activation barrier (figure 2). If the addition reaction is barrierless, the rate constant only depends on a frequency factor (probability of a favorable event in which F^+ and CH_3CN collide with the appropriate geometry). Once the event is realized, the energy corresponding to the formation of the $[R–C_6H_4CH_2^+]–[NC–CH_3]$ bond (= E_b) is redistributed on the complex, and the energy in the reaction coordinate drops, "diluted" in the other degrees of freedom, resulting in stabilization of the complex. The (barrierless) collision rate constant is given either by the Langevin [11,12] or ADO [13-15] theory, considering the ion as a point charge. The neutral is either a dipole (ADO) or a polarizable molecule (Langevin). Both theories result in a rate constant directly proportional to the charge q of the ion [13]. In our case, the benzyl ion can not be considered as a point charge, as the charge is delocalized on the aromatic ring and the substituent. Therefore, the latter strongly influences the effective charge q_{eff} on the α-carbon atom of the benzyl ion. The more electron withdrawing the substituent, the higher the charge density (q_{eff}) on the benzylic carbon atom, and the higher the collision (association) rate constant.

2. *Observation of acetonitrile adducts in full scan ES-MS*

In the case of full-scan ES mass spectra on the LCQ instrument, there is an inherent ambiguity on where reaction 2 occurs. At least part of the amount of adduct detected in full-scan mass spectra is due to acetonitrile addition in the trap, but the injection/ejection process does not take more than tens of milliseconds with the chosen mass range, and the kinetics of addition in the trap is too slow to account for the total adduct intensity (see table 1) observed in the full scan spectra. Addition must also have occurred in the source (p 1 torr) or in the transfer octopoles (p 10^{-3} torr). With the Zabspec-T, the situation is even clearer: in order to be detected, the adducts must have been formed before the first electric sector, probably in the sampling cone-skimmer region (rotary pumped) or in the hexapole region (p 10^{-3} mbar). The formation of Add^+ ions is attributed to the two-step (S_N1-like) mechanism described in figure 1 for two reasons: (1) an S_N2-like mechanism is highly improbable due to the higher gas-phase basicity of pyridine compared to acetonitrile and (2) the constant ratio between $I(Add^+)$ and $I(F^+)$ throughout the source-CID experiment (figure 4) is consistent with the formation of Add^+ from F^+. The significant amount of adduct detected, especially with the Zabspec-T, indicates that ions are fragmenting quickly enough in the electrospray source so that the fragments have time to make a quite large number of collisions to interact with the vaporized electrospray solvent.

Conclusion :

This study illustrates that the interaction of electrospray-produced desolvated ions with residual solvent vapors in the plume, or more downstream the mass spectrometer, can be of significant importance for the appearance of the mass spectra. Detailed quantitative studies of these reactions in the electrospray source are however difficult due to the absence of control on the time window (imposed by the instrument) and on the partial pressures (due to differential pumping). The quadrupole ion trap is much better suited for the study of ion-molecule reactions with its wide possibilities to vary the sequence of events. In the particular case presented here, the neutral is simply the electrospray solvent, provided by leakage from the source. Improvements have of course still to be made to enable the measurement of the partial pressure and to vary it in a controlable and reproducible manner, but this preliminary study points to a wide variety of applications to the study of solvation reactions by ES-QIT mass spectrometry.

References :

1. C. Collette and E. De Pauw, Rapid Commun. Mass Spectrom., 12 (1998) 165.
2. C. Collette, L. Drahos, E. De Pauw and K. Vékey, Rapid Commun. Mass Spectrom., 12 (1998) 1673.
3. L. Drahos, R.M.A. Heeren, C. Collette, E. De Pauw and Vékey, K. J. Mass Spectrom. 34 (1999) 1373.
4. F. Derwa, E. De Pauw and P. Natalis, Org. Mass Spectrom., 26 (1991) 117-118.
5. A.R. Katritzky, C.H. Watson, Z. Dega-Szafran and J. Eyler, J. Am. Chem. Soc., 112 (1990) 2471.
6. E. Anders, R. Koch, A.R. Katritzky, N. Malhotra, J. Eyler and J.A. Zimmerman, Chem. Ber., 125 (1992) 177.
7. L.P. Hammett, J. Am. Chem. Soc., 59 (1937) 96.
8. H.C. Brown and Y. Okamoto, J. Am. Chem. Soc., 80 (1958) 4979.
9. A.G. Harrison, J. Mass Spectrom., 34 (1999) 577.
10. A.G. Harrison, P. Kebarle, and F.P. Lossing, J. Am. Chem. Soc., 83 (1961) 777.
11. M.P. Langevin, Ann. Chim. Phys., 5 (1905) 245.
12. G. Gioumousis and D.P. Stevenson, J. Chem. Phys., 29 (1958) 294.
13. T. Su and M.T. Bowers, J. Chem. Phys., 58 (1973) 3027.
14. T. Su and M.T. Bowers, Int. J. Mass Spectrom. Ion. Phys., 12 (1973) 347.
15. L. Bass, T. Su, W.J. Chesnavich and M.T. Bowers, Chem. Phys. Lett., 34 (1975) 119.

Tables :

Table 1: Summary of the results obtained for the benzyl cations with different substituents –R, and of the σ^+ parameters for *meta* and *para* substituents.

	–R	**$I(Add^+)/I(F^+)$**[a]		**$k.[CH_3CN]$ (s^{-1})**[b]	**σ^+ [8]**
		Zabspec-T	**LCQ**		
1	p-NO_2	-	0.82	5.88 10^{-1}	0.790
2	m-CN	-	0.31	4.02 10^{-1}	0.562
3	p-CN	-	0.31	3.48 10^{-1}	0.659
4	m-F	-	0.10	1.43 10^{-1}	0.352
5	o-F	-	0.06	6.72 10^{-2}	-
6	m-OCH_3	0.68	0.02	4.59 10^{-2}	0.047
7	m-CH_3	0.35	0.012	-	-0.066
8	p-F	0.22	< 0.01	-	-0.073
9	p-Cl	0.19	< 0.01	-	0.114
10	o-CH_3	0.11	< 0.01	-	-
11	p-CH_3	0.03	< 0.01	-	-0.311
12	p-OCH_3	0.00	< 0.01	-	-0.778

[a]Ratio of the intensities obtained for the source-CID experiments on the two instruments (mean of 5 values obtained at different sample cone voltages (ZabSpec-T) or capillary voltages (LCQ)).

[b]Observed reaction rate constant k_{exp} obtained from the slope of the linear regressions of figure 6.

Figure Legends :

Figure 1: Structure of the benzylpyridinium, benzyl, adduct ions and reaction scheme for the dissociation of benzylpyridinium ions and reaction of benzyl cations with acetonitrile.

Figure 2: Potential energy surface along the reaction coordinate for the dissociation of a F^+–Nu ion-neutral complex, which can be either M^+ (nucleophile = pyridine) or Add^+ (nucleophile = acetonitrile). It is assumed that there is no reverse activation energy barrier.

Figure 3: ESI-MS full scan spectra obtained on the ZabSpec-T for m-OCH_3, m-CH_3, p-CH_3 and p-OCH_3 benzylpyridinium ions (see figure 1 for the annotation conventions). The voltage difference between the sampling cone and the skimmer was 67.2 V, except for p-OCH3 (28.8 V).

Figure 4: relative intensities of M^+, F^+ and Add^+ in the spectra of m-CH_3 benzylpyridinium ion as a function of the voltage difference between the sampling cone and the skimmer (Zabspec-T instrument).

Figure 5: Kinetics of the addition reaction: fraction of the total intensity as a function of the time for the three most reactive benzyl ions. F^+ was produced by MS^2 on M^+, selected in MS^3, and stored in the trap (activation amplitude = 0%, q_z = 0.25) for different amounts of time. The intensities are normalized to $I(Add^+) + I(F^+)$.

Figure 6 : Determination of the association rate constants for the six most reactive benzyl cations: (1) p-NO_2, (2) m-CN, (3) p-CN, (4) m-F, (5) p-F and (6) m-OCH_3. The slopes give access to the k_{exp} = k.[CH_3CN] values that are given in table 1.

Figure 7: Influence of the flow rate on the kinetics of acetonitrile addition on m-CN benzyl ion. The rate constants were obtained by measuring the slope of the linear regression of $-\ln[I(F^+)/I(F^+)+I(Add^+)]$ as a function of the time at each flow rate.

Figure 8 : Correlation between the observed rate constants (determined from figure 6) and the σ^+ [8] parameters.

Figure 1

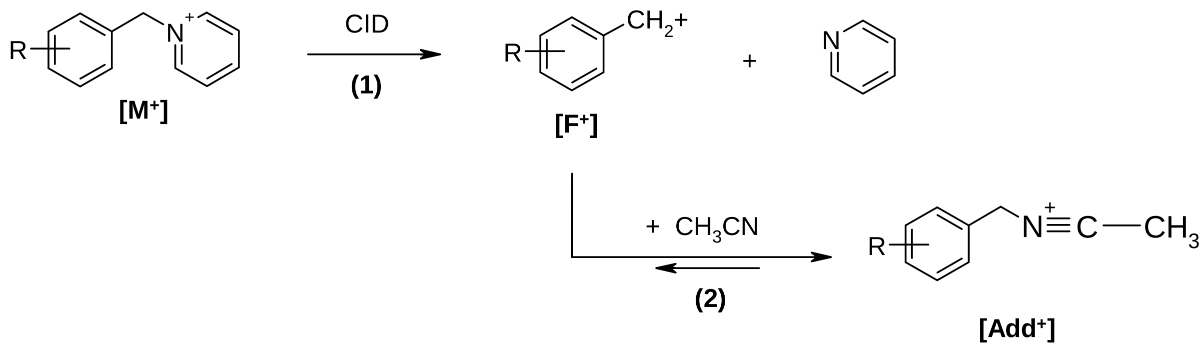


Figure 2

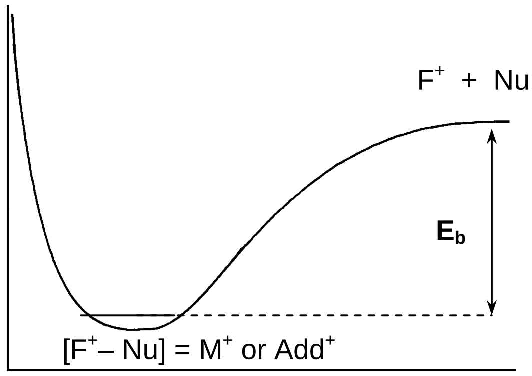
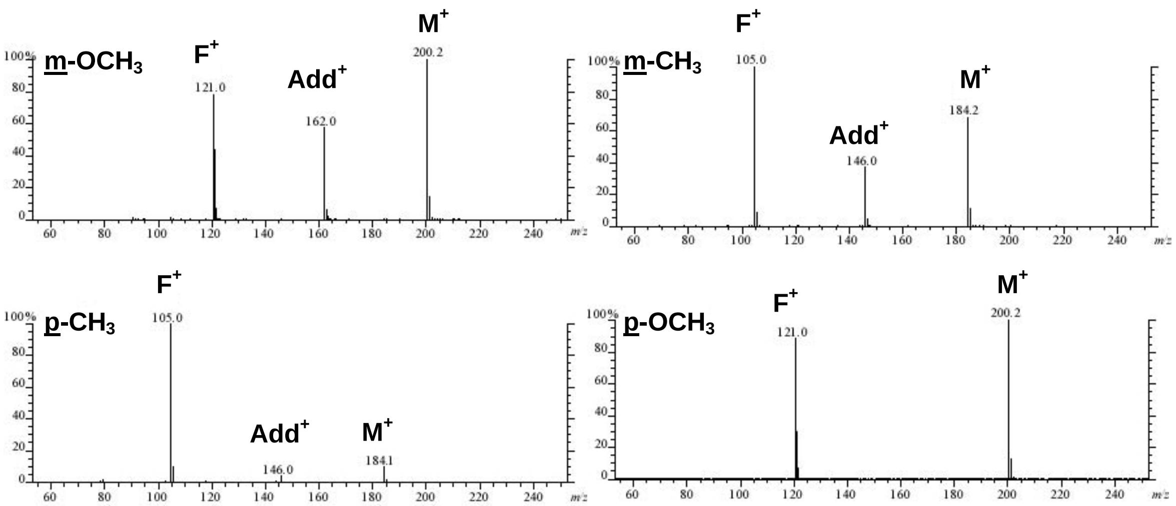
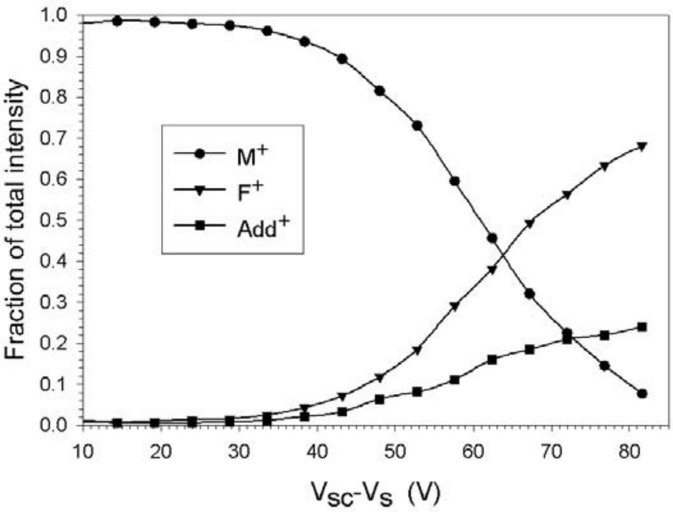


Figure 3



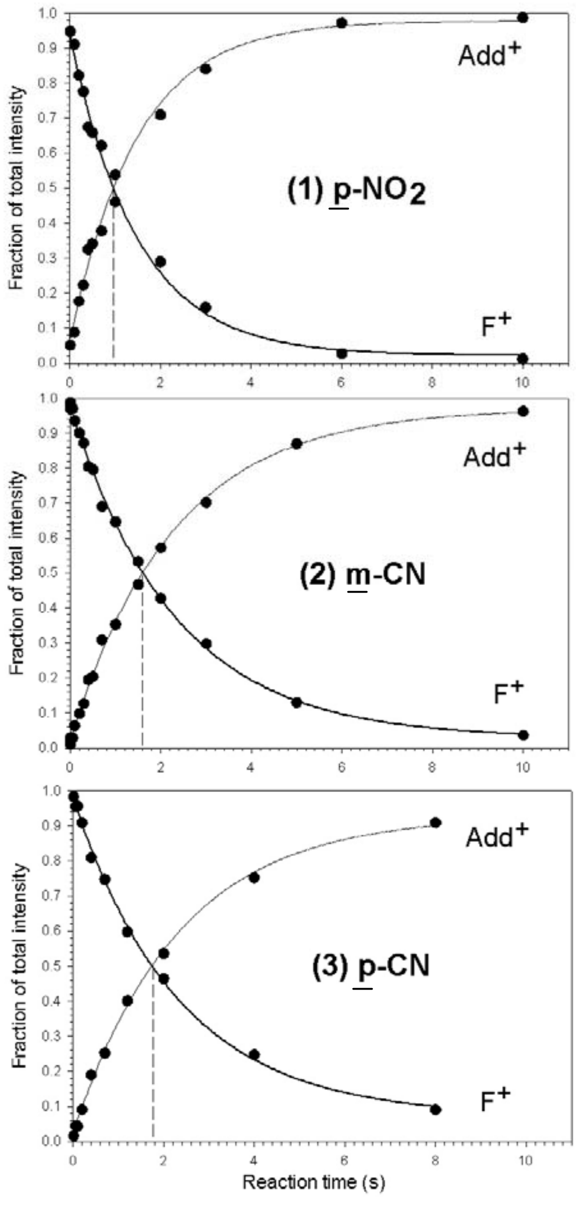
m-OCH3
F+
121.0
Add+
162.0
M+
200.2
m-CH3
F+
105.0
Add+
146.0
M+
184.2
p-CH3
F+
105.0
Add+
146.0
M+
184.1
p-OCH3
F+
121.0
M+
200.2
m/z

Figure 4



1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Fraction of total intensity
M+
F+
Add+
10
20
30
40
50
60
70
80
VSC-VS (V)

Figure 5



Add+
(1) p-NO2
F+
Add+
(2) m-CN
F+
Add+
(3) p-CN
F+
Fraction of total intensity
Reaction time (s)

Figure 6

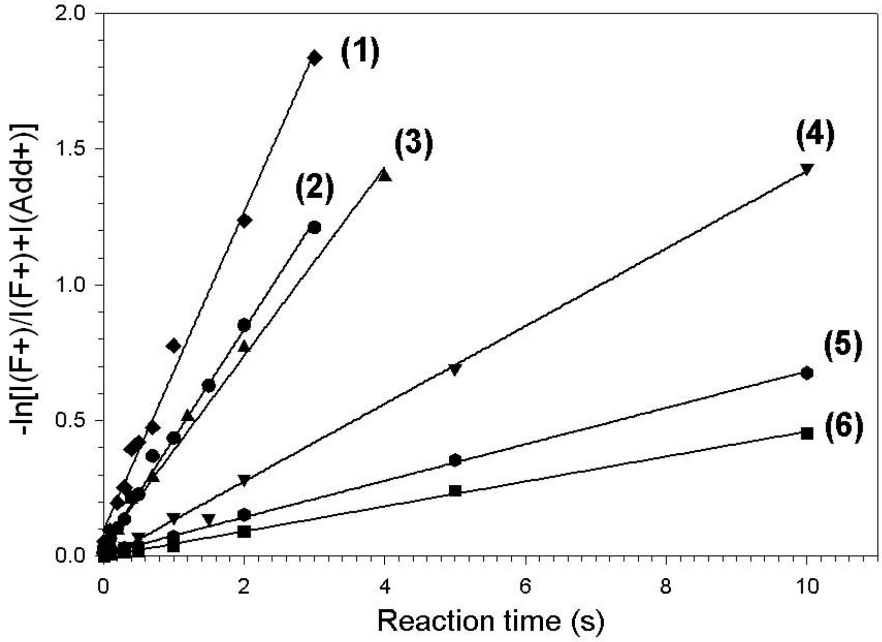
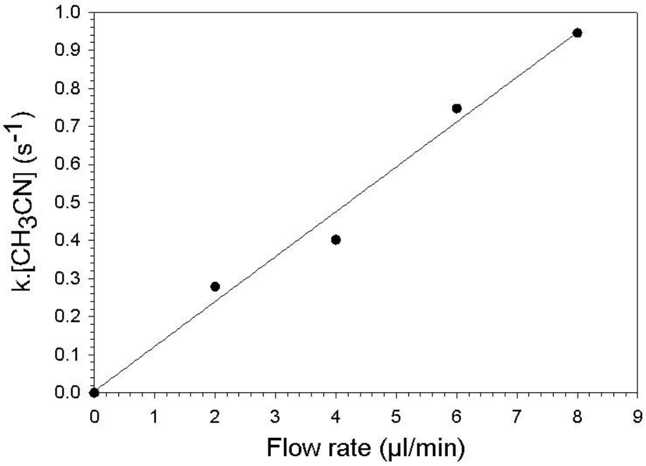
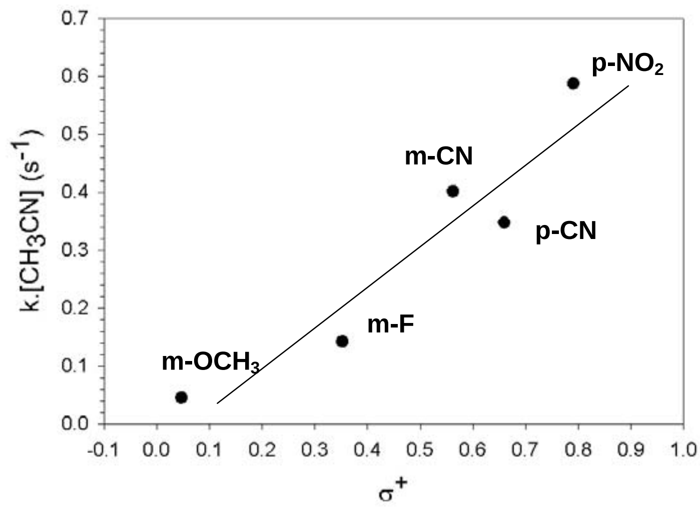


Figure 7



k.[CH3CN] (s-1)
Flow rate (µl/min)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9

Figure 8



0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
k.[CH3CN] (s-1)
-0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
σ+
p-NO2
m-CN
p-CN
m-F
m-OCH3