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# Non-thermal internal energy distribution of ions observed in an electrospray source interfaced with a sector mass spectrometer

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The internal energy distribution  $P(E_{int})$  of ions emitted in an electrospray (ESI) source interfaced with a sector mass spectrometer is evaluated by using the experimental survival yield (SY) method including the kinetic shift. This method is based on the relationship between the degree of fragmentation of an ion and its amount of internal energy and uses benzylpyridinium cations due to their simple fragmentation scheme. Quantum chemical calculations are performed, namely at G3(MP2)//B3LYP and QCISD/MP2 levels of theory. The results show that the internal energy distribution of the ions emitted in the ESI source interfaced with a sector analyzer is very narrow. The MassKinetics software is used to confirm these observations. The  $P(E_{int})$ is the parameter that allows to fit the experimental SY of each substituted benzylpyridinium cation with theoretical mass spectra generated by the MassKinetics software. The resulting internal energy distributions are similar to the ones obtained with the experimental SY method. This indicates that in the present experimental conditions,  $P(E_{int})$  cannot be compared with a 'thermal-like' Boltzmann distribution. In addition, it appears that with the sector analyzer, increasing the collision energy in the first pumping stage of the ESI source does not correspond to a warm-up of the produced ions. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: electrospray ionization; internal energy; RRKM; sector mass spectrometer; thermometer ions

## Introduction

The mass spectra of fragment ions produced in an ion source depend on the internal energy distribution  $P(E_{int})$  of the precursor ions, the energetic barrier of the unimolecular reaction and the time window corresponding to the observation of the dissociation process.<sup>[1,2]</sup> In electron impact (EI) ionization, a ionizing electron kinetic energy of 70 eV leads to a typical internal energy distribution that lies until  $\sim$ 16 eV with a maximum around 4 eV.<sup>[1]</sup> It is admitted that this distribution is reproducible whatever the used instrument due to the low pressure of the source and the ion acceleration zone. The El mass spectra can then be compiled to create databases. For the other ionization modes, the energetic characteristics of the produced ions are less well defined than for El. Among these methods, electrospray ionization (ESI) is becoming one of the most used ionization modes.<sup>[3]</sup> Using ESI sources, the ions are emitted in the gas phase after their formation in solution and will cross regions of more or less high pressures before entering in the mass analyzer.<sup>[4-6]</sup> In these regions, their internal energy can be modified depending on different experimental conditions related to the geometry and the operation parameters of the interface (temperature, pressure, applied voltages, etc.). The transport of ions from the ion source into the interface requires small apertures to separate the atmospheric pressure from the vacuum of the interface.<sup>[7]</sup> This orifice produces a well-defined supersonic jet in expansion in which ions and gas are accelerated to supersonic velocities.<sup>[8]</sup> This barrel-shaped expanding jet terminates in a shock wave also referred to as the Mach disc.<sup>[9]</sup> The ions carried by the expanding gas are usually sampled into the next vacuum stage through a

skimmer. As the skimmer tip is located upstream of the Mach disc, the velocity of the ions is high with an axial component increased towards the radial velocity and with low gas density in this so-called silence zone. If the skimmer is positioned outside the Mach disc, the pressure is higher but the gas velocity is lower.<sup>[10]</sup> In most cases, additional focusing items are introduced between the orifice and the skimmer in order to achieve a good efficiency in the ion sampling, but they redefine both the internal and the

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translational energies of the electrosprayed ions.[11] Indeed, if the desolvation process can be performed in the atmospheric pressure side by drying the ions with heat or dry gas, the removing of the last solvent molecules is rather achieved in the vacuum side by means of collision-induced dissociation (CID). This process is performed by in-source CIDs (in-source CID) with the residual gas and controlled by the application of voltage differences between the extraction lenses of this 'nozzle-skimmer region'.<sup>[12,13]</sup> In this first pumping stage, the influence of pressure on the transmission efficiency of ions<sup>[14]</sup> and the desolvation conditions has been described.<sup>[15,16]</sup> The production of net ions from a supra-thermal-like desolvation process can also be in competition with ion fragmentation due to collisional activation. In the case of source decays, the in-source CID mass spectra are then considered as an additional tool for structural determination.<sup>[17-19]</sup> However, before the entrance in the mass analyzer, the ions also pass through a second pumping stage where the pressure is dramatically lowered. This region is designed for ion transmission by means of ion guides (quadrupole or hexapole lens). Due to the main free path of ions, this zone has been described as having high collision efficiency.<sup>[20]</sup> Increasing the pressure in this region is also considered as having a strong beneficial effect on the ion signal intensity.<sup>[21]</sup>

For constructing an ESI in-source CID mass spectra library, it is then important to calibrate in internal energy the recorded mass spectrum.<sup>[22]</sup> The results reported in the literature show that the internal energy distribution  $P(E_{int})$  of the ions in an ESI source can be compared with 'thermal-like' Boltzmann distributions related to a simple parameter called the 'characteristic temperature'  $(T_{char})$ .<sup>[23-25]</sup> The method proposed for evaluating this internal energy distribution was referenced by De Pauw and coworkers as the 'survival yield' (SY) method.<sup>[26]</sup> This one can be described on the basis of relationship between the degree of fragmentation of an ion and its amount of internal energy.<sup>[27]</sup> The SY method used namely substituted benzylpyridinium cations due to their simple fragmentation scheme that leads to the loss of neutral pyridine and the formation of a substituted benzyl cation.<sup>[28]</sup> The experimental measurement of ion SY has been performed from ESI sources interfaced with different analyzers, such as guadrupoles,<sup>[25-27]</sup> Fourier transformation ion cyclotron resonance cells<sup>[29]</sup> and time-of-flight mass spectrometers.<sup>[30]</sup> All these sources use nitrogen as nebulizing and dry gas leading to thermal-like internal energy distribution when the desolvation conditions are properly adjusted.<sup>[31,32]</sup> An empirical equation has been recently proposed for evaluating the mean internal energy of the ions emitted in an ESI source. This equation takes into account different parameters such as the cone voltage value, the source temperature, the ion degree of freedom and the ion mean internal energy considered from the source temperature at a zero cone voltage value.<sup>[33]</sup> Tabet and coworkers have also shown that the De Pauw method can be combined with the use of the MassKinetics software of Drahos and Vékey in order to evaluate the internal energy distribution of gaseous ions generated in some ESI sources.<sup>[34]</sup> With the MassKinetics software, a theoretical SY has been calculated from different species (benzylpyridinium cations, aromatic benzoic esters and peptides) and for different internal energy distributions.<sup>[35]</sup> However, at our knowledge, if the reactivity of the benzylpyridinium has been studied with an electrospray source interfaced with a sector mass spectrometer, no result has been reported in the literature concerning an internal energy distribution evaluated under these conditions.<sup>[36]</sup>

In this study, an electrospray source interfaced with a JEOL sector mass spectrometer was used. This source acts without the

use of dry gas. On one hand, the internal energy distribution of ions emitted was evaluated using the experimental SY method of De Pauw *et al.* by including the kinetic shift. On the other hand, the theoretical SYs of the thermometer ions were calculated by using the MassKinetics software. This one generates theoretical mass spectra by considering the internal energy distribution of the ions as an adjusting parameter. In order to point out the important parameters to be considering for the evaluation of  $P(E_{int})$ , the principles of the SY method are first described in this paper.

#### **Methods**

The survival yield SY of the parent ion  $M^+$  is related to the abundance of the parent ion  $I_M^+$  and the fragment ions  $I_F^+$  measured from the recorded ion source mass spectra such as

$$SY = \frac{I_{P^+}}{\left(I_{P^+} + \sum_{F^+=1}^n I_{F^+}\right)}$$
(1)

This is the fraction of the parent ions that have an internal energy below the appearance energetic threshold  $E_{thr}$  of the fragment ions. (Note that for the present paper the denomination 'appearance energetic threshold' is preferred to the term appearance energy, this one being rather defined as the minimum ionizing electron energy necessary to observe a fragment ion in El/MS.)



**Figure 1.** Illustration of the procedure used in the survival yield method (SY) including the kinetic shift (ks) for evaluating of the ion internal energy distribution from the reactivity of different substituted benzylpyridinium cations. (a) Evolution of the logarithm of the dissociation rate constant of cation selected for the study as a function of their internal energy ( $E_{int}$ ). (b) Experimental survival yield of thermometer ions (\*) reported as a function of appearance threshold energy ( $E_{app}$ ) calculated from the residence time ( $\tau$ ) of ions in the ESI source. (c) lon internal energy distribution ( $P(E_{int})$ ) obtained from the derivate of the curve plotted from a fit of the data in (b) with a sigmoid function. Note that this illustration shows also the addition of the two points (x) required for the fit with a sigmoid curve at SY = 0 and SY = 1.

As illustrated in Fig. 1, the different values of SY are plotted as a function of the 'appearance energetic threshold'. Two points are added manually by considering that all the precursor ions are fragmented if  $E_{thr} = 0$  and that all the parent ions survive at a high values of  $E_{thr}$ . The fitting of the points with a sigmoid curve allows to obtain the internal energy distribution *via* the derivation of the SY( $E_{int}$ ) curve. The value of  $E_{thr}$  is obtained from the kinetic shift ( $E_{ks}$ ) that represents the excess of energy by comparison to the critical energy of the reaction  $E_0$ , necessary to fragment the ions at observable rates, such as

$$E_{\rm ks} = E_{\rm thr} - E_0 \tag{2}$$

It has been shown that  $E_{\rm ks}$  can be calculated using the Rice–Ramsperger–Kassel–Marcus (RRKM) theory.<sup>[37–39]</sup> This one allows to express the rate constant of an ion fragmentation reaction at a given internal energy  $E_{\rm int}$  such as

$$k(E_{\rm int}) = \frac{\sigma}{h} \frac{N^{\ddagger}(E_{\rm int} - E_0)}{\rho(E_{\rm int})}$$
(3)

In Eqn (3),  $\sigma$  is the reaction degeneracy, *h* is the Planck's constant,  $E_0$  is the reaction critical energy,  $N^{\dagger}(E_{int} - E_0)$  is the transition state sum of states from 0 to  $E_{int} - E_0$  and  $\rho(E_{int})$  is the density of states at an energy level equal to  $E_{int}$ .<sup>[2,40]</sup> The evaluation of the sum and density of states is usually done by a direct count using the Beyer-Swinehart algorithm that uses frequency models obtained by theoretical calculation from the reactant and the activated complex at the transition state of the reaction.<sup>[41]</sup> The fundamental assumption of the experimental SY method is that the fragmentation of the benzylpyridinium cations  $PyR^+$ occurs only via a direct cleavage of the C-N bond between the substituted benzyl group and the pyridine Py. Even though recent theoretical and experimental systematic studies have shown that fragmentations with rearrangement can compete with the direct cleavage,<sup>[42-44]</sup> the SY method considers that the dissociation of the benzylpyridinium ions takes place without any reverse activation energy (Fig. 2). The absence of reverse energy implies that the critical energy  $(E_0)$  for the fragmentation of a

benzylpyridinium ion is equivalent to the value of  $\Delta(\Delta H_{\rm f})$  for the reaction, such as

$$E_0 = \Delta(\Delta H_f) = \{ [\Delta H_f(\mathsf{Py}) + \Delta H_f(R^+)] - \Delta H_f(\mathsf{Py}^+R) \}$$
(4)

(Note that this equation does not consider that critical energy is at 0 K, while the enthalpy is usually room temperature. The critical energy can be, however, determined from the standard enthalpy, which can be measured easily, using a set of equations developed by Pople and coworkers.<sup>[45]</sup>) In addition, with these 'thermometer' ions, the evaluation of the internal energy deposited on the precursor ion is based on the following assumptions:

- any ionization energy has to be taken into account in the production of the gaseous ions since the ions emitted into the gas phase result from a charge separation in solution;
- (2) the internal energy is of vibrational mode for all the ions;
- (3) the ions have similar mass, structures, degree of freedom number and center of mass collision energy;
- (4) the intake of internal energy per vibrational mode is the same for all the studied ions in the same experimental conditions due to the thermal equilibrium conditions in an atmospheric pressure source.

The first studies reported in the literature referred to either published  $\Delta H_{\rm f}$  values or  $E_0$  values that were correlated from the ionization energies of the corresponding substituted benzyl radicals.<sup>[26,27]</sup> The critical energy values have also been evaluated from semi-empirical (AM1) or chemical quantum calculation at different theoretical levels (*ab initio* HF/6-31G<sup>\*</sup> and DFT B3LYP/6-31G).<sup>[25]</sup> The variations between the predicted values have shown the importance of the choice of the method and the level of calculation.<sup>[11]</sup>

The appearance energetic threshold  $E_{thr}$  of each substituted benzylpyridinium cation is obtained through the relation between the logarithm of the rate constant and the corresponding internal energy, the value of  $k(E_{int})$  being the reciprocal of the residence time of ions in the source (Fig. 1). Note that the value of  $E_{thr}$  is related to different theoretical and experimental parameters that can be listed as follows:



**Reaction Coordinate** 

**Figure 2.** Dissociation scheme of the substituted benzylpyridinium cations and potential energy evolution of the fragmentation as a function of the C–N central bond distance considered as the reaction coordinate. The parameters  $E_0$  and ks are related to the critical energy and the kinetic shift of the reaction.

- (1) the critical energy of the reaction  $E_0$  and the molecular frequencies of the reactant ion obtained from the theoretical calculation, their values being related to the level of calculation;
- (2) the magnitude of  $k(E_{int})$  curve that is a function of the frequencies of the reaction transition and ground states, its evaluation being often characterized through the looseness of the transition state;
- (3) the residence time of ion in the ESI interface that determines the ks.

# **Experimental Section**

#### Mass spectrometry experiments

Mass spectrometry analyses were performed on a JMS-700 (Jeol Ltd, Akishima, Tokyo, Japan) double focusing mass spectrometer with reversed geometry, equipped with a pneumatically assisted ESI source. Nitrogen was used as the nebulizer gas. Positive ion mode was used with a needle voltage adjusted in order to obtain a  $\sim$ 500 nA needle current (*i*<sub>ESI</sub>), i.e. typically  $\sim$ 3 kV. The electrospray source and the optical path of the JMS 700 are schematically described in Fig. 3. The potential difference between the desolvating plate and the first skimmer, i.e. the orifice 1 or OR1, can be varied from 0 to 300 V. Three desolvating plate temperature values were chosen for the study, i.e. 423, 473 and 523 K, the orifice 1 temperatures being then stabilized as a function of these temperatures, i.e. 353, 361 and 371 K, respectively. No voltage was applied to the ring lens (RL in Fig. 3). This one is used to collect the ions along the center axis of the source. Three parts are particularly depicted in Fig. 3. The first indicates the skimmer region in the first pumping stage. A rough pump (650 l min<sup>-1</sup>) is used to evacuate the first region to about 2 Torr. The second part is related to the transfer quadrupole zone in which the pressure is decreased to above 10<sup>-3</sup> Torr by means of a turbomolecular pump. The last part corresponds to the last pumping stage that limits the region of ion acceleration undergoing a potential difference of 5000 V at a pressure close to that of the analyzer  $(10^{-6} \text{ Torr})$ . In the case of Fig. 3, a 120-V potential difference between the skimmer and the counter electrode is set, but a 90-V voltage value was also used in the study as indicated in the Section on Results and Discussion. This potential difference is also called the cone-voltage value (CV).

#### Chemicals

The substituted benzylpyridinium salts (Table 1) were prepared by condensation of the substituted benzyl halide with pyridine. The salts precipitated after a few hours of reflux heating and were recrystallized with diethyl ether. Their structure was confirmed by NMR spectroscopy.

The benzylpyridinium salts depicted in Table 1 were dissolved in a CH<sub>3</sub>OH/H<sub>2</sub>O (1:1) solvent mixture at a 5 × 10<sup>-5</sup> M concentration. Two solutions were separately injected. The first contained the *N*-(4-methoxybenzyl)pyridinium ion (*p*-CH<sub>3</sub>O), the *N*-(4-methylbenzyl)pyridinium ion (*p*-CH<sub>3</sub>), the *N*-(benzyl)pyridinium ion (H) and the *N*-(4-fluorobenzyl)pyridinium ion (*p*-F). The second contained the *N*-(3-methoxybenzyl)pyridinium ion (*m*-CH<sub>3</sub>O), the *N*-(3-methylbenzyl)pyridinium ion (*m*-CH<sub>3</sub>) and the *N*-(4nitrobenzyl)pyridinium ion (*p*-NO<sub>2</sub>).

#### **Quantum chemistry**

All gas-phase calculations were carried out with the Gaussian03 suite of programs.<sup>[46]</sup> Owing to the large number of possible reactions, rapid explorations of the potential energy surfaces (PES) have been performed using HF and B3LYP methods in conjunction with the 6-31G\* basis set. Significantly different results were obtained for the direct C–N bond cleavage of several substituted benzylpyridinium cations. This has led us to consider small model systems which will be presented in the Section on Results and Discussion. For the model systems, we have realized fully relaxed scans for C–N bond breaking as well as geometry optimizations for the stationary points. HF, MP2, CISD and B3LYP methods have been retained for this purpose in combination with the  $6-31+G^{**}$ 



**Figure 3.** Schematic representation of the ESI source and optical path of the JMS 700 mass spectrometer, showing the distance between the different parts of the interface that is considered for the calculation of the residence time and the applied voltages. Note that for the present example, a 120-V potential difference is considered in the declusterization area delimited by the lenses OR1 and OR2.

# MASS SPECTROMETRY

Table 1. Substituted benzylpyridinium salts used for the study						
Structure	Compound name	Cation name $(m/z)$				
MeO N O CI	N-(3-methoxybenzyl)pyridinium chloride	<i>m</i> -CH <sub>3</sub> O (200)				
O <sub>2</sub> N N ⊕ Br	<i>N</i> -(4-nitrobenzyl)pyridinium bromide	<i>p-</i> NO <sub>2</sub> (215)				
$ \begin{array}{c} Br \\ & \\ N \\ \oplus \\ Br \end{array} \\ \\ \\ Br \end{array} $	<i>N</i> -(4-bromobenzyl)pyridinium bromide	<i>p</i> -Br (248)				
Me N Br	<i>N</i> -(3-methylbenzyl)pyridinium bromide	<i>m</i> -CH <sub>3</sub> (184)				
Me N ⊕ Br	<i>N</i> -(4-methylbenzyl)pyridinium bromide	<i>р-</i> СН <sub>3</sub> (184)				
MeO N ⊕ CI	N-(4-methoxybenzyl)pyridinium chloride	<i>p</i> -CH₃O (200)				
F N Br	<i>N</i> -(4-fluorobenzyl)pyridinium bromide	<i>p-</i> F (188)				
⊖ N ⊖ Br	Benzylpyridinium bromide	BzPyr (170)				

basis. In the case of substituted benzylpyridinium cations and theirs C–N bond cleavage products, the geometries, energies and vibrational properties were recomputed at the B3LYP/6-31+G<sup>\*\*</sup> and MP2/6-31+G<sup>\*\*</sup> levels of theory. In particular, the harmonic frequencies were computed in order to characterize the stationary points (minima vs transition state structures (TS)) and to estimate theirs thermodynamic parameters. Gibbs energy of dissociation has been estimated at a 350 K temperature and a 1 atm pressure according to the reaction described in Scheme 1.

Note that some of the studied species exhibit several conformers. Hence, Gibbs energies of these species have been evaluated through a Boltzmann distribution according to relation (5)

$$G^{0}_{\{A\}} = -RT \ln \sum_{i \in \{A\}} e^{-G_{i}/RT}$$
(5)



**Scheme 1.** Unimolecular reaction of the substituted benzylpyridinium cations.

where  $\{A\}$  emphasizes calculation over the population of all conformers of A. Gibbs energy of the reactions was then refined by using the results of G3(MP2)//B3LYP and QCISD/6-31+G\*\* calculations. G3(MP2)//B3LYP is a modification of G3 extrapolation procedure that requires significantly less computational time.<sup>[47]</sup> The average absolute deviation from experiment of G3(MP2)//B3LYP method for a total of 299 energies (enthalpies of formation, ionization potentials, electron affinities, proton affinities) is 1.25 kcal mol<sup>-1</sup>. As bromo-substituted species could not be studied with G3(MP2)//B3LYP method, total energies of the various species were recomputed at the QCISD/6-31+G\*\* theoretical level using previously MP2/6-31+G\*\* optimized structures. For the sake of simplicity, Gibbs energies calculated using QCISD/6-31+G\*\*//MP2/6-31+G\*\* energies and MP2/6-31+G\*\* structural and vibrational properties will be referred to QCISD//MP2 throughout the text. Note that the core electrons were frozen for all post-HF calculations.

# Theoretical modeling of reaction rate constants and mass spectra

The fragmentation rate constants and the theoretical SY of the substituted benzylpyridinium cations were calculated using the MassKinetics Scientific (Ver. 1.9) software, available at http://www.chemres.hu/ms/masskinetics. MassKinetics is a general framework for modeling mass spectrometric processes.<sup>[35]</sup> It combines modeling reaction rates, energy exchanges and product ion abundances as the ions move through various parts of a mass spectrometer. In MassKinetics, the ions are characterized by their internal and kinetic energies, which define the 'state' of an ion. Essential features of the MassKinetics model are the use of internal (and, if necessary, kinetic) energy distributions and the use of probabilities to describe transition between different states. Reaction rates are calculated via the transition state theory (TST)<sup>[48,49]</sup> in its RRKM formulation.<sup>[37-40,50,51]</sup> The kinetic and/or internal energies of ions in the gas phase may change due to (1) acceleration in electromagnetic fields, (2) radiative energy exchange (photon-absorption and emission), (3) collisional energy exchange and (4) energy partitioning in chemical reactions, which are all taken into account. With MassKinetics, the ion abundances and thus the theoretical SY can be calculated accurately using no or only very few empirical (adjustable) parameters. MassKinetics modeling requires the vibrational frequencies of each substituted benzylpyridinium cation obtained from theoretical calculation (vide infra), and the harmonic oscillator model is applied with internal rotations approximated by low-frequency vibrations. In the present work, the internal energy distribution has been considered as an adjustable parameter. As a result, the theoretical SY of each studied benzylpyridinium cation was fitted against the experimental SY by using either a thermal-like internal energy distribution or an internal energy distribution from on adjusted Gaussian function. This distribution is supposed to be characteristic of a gaseous ion population sampled in front of the orifice 2 (Fig. 3).

The kinetic energy of the species prior to their acceleration is related to the potential difference between the orifices 1 and 2, e.g. 120 V in the case of Fig. 3. The MS experimental sequence fixed for the calculation of a theoretical SY from an ESI ionization interfaced with the JMS 700 Jeol magneto-electrostatic mass spectrometer is (1) the ion formation without any internal energy deposited during this process; (2) a field-free flight from the orifice 2 to the orifice 3 with a 0.28720 m flight length (Fig. 3), (3) an electrostatic acceleration under a 5 kV potential difference with a 1.6-cm flight length and (4) the ion selection followed by the detection (from the acceleration lens to the detector) with a 1.9656 m flight length. The critical energy of the reaction has been calculated using the G3(MP2)//B3LYP or the QCISD//MP2 methodologies (vide supra). The frequency model of the ground state that allows to express  $\rho(E_{int})$  is obtained from the vibration frequency values calculated at the G3(MP2)//B3LYP or the QCISD//MP2 levels of theory. It is more difficult to evaluate the vibration frequencies of the activated complex that lead to the sum of state  $N^{\ddagger}(E_{int} - E_0)$  in Eqn (4). In the case of the fragmentation of the benzylpyridinium ions, one defines a 'loose' complex corresponding to a direct cleavage due to a stretching of the C-N bond along the reaction coordinate. The 'looseness' of the transition state can be characterized by the Arrhenius pre-exponential-like factor A<sub>PE</sub> that takes into account the frequency models of the reactants and the transition states through the expressions of the vibration entropy differences. For the substituted benzylpyridinium fragmentation, the expected A<sub>PE</sub> value is considered to be close to 14.<sup>[1,34,52,53]</sup> In this context, the frequency file of the transition state is calculated from the frequency model of the reactants as follows: the frequency corresponding to the elongation of the C–N bond (1200  $cm^{-1}$ ) is removed and five other frequencies are scaled to obtain the expected  $A_{\rm PE}$  value, i.e. two at ~800, two at ~600 and one at  $\sim$ 100 cm<sup>-1</sup>. This is a convenient way for simulating the transition state.<sup>[54,55]</sup>

# **Results and Discussion**

#### **Chemical quantum calculation**

In the present study, we have investigated the mechanism of the direct bond cleavage between N and C atoms of the substituted benzylpyridinium cations. It appeared for several benzylpyridinium ions that the consideration of a transition state along the reaction coordinate during the C–N bond stretching depends on the level of theory used: HF/6-31G\* or B3LYP/6-31G\*. This result prompted us to consider model systems to assess the underlying mechanisms of the effective dissociation process.

## Mechanism of the direct C-N bond cleavage

In order to reach a reasonable level of theory, we have selected three relatively small model systems (Scheme 2). The chosen model systems allow us to simulate with a reasonable accuracy the structural variations of the real systems. Figure 4 displays the energy variation of the system 1 computed at various levels of theory when the C-N bond dissociation proceeds (all other geometrical parameters being relaxed). We observed that the whole ab initio calculations predict the dissociation without any potential energy barrier to form CH<sub>3</sub><sup>+</sup> and NH<sub>3</sub> species. In contrast, at B3LYP/6-31+G\*\* level of theory, the dissociation mechanism involves a TS located at  $d_{C-N} = 6.0$  Å. A closer examination of the corresponding dissociation curve reveals two highly abnormal features that should be pointed out: (1) the total energy of the fragments produced by the bond cleavage is not identical to, but much lower than, the sum of the total energies of the NH<sub>3</sub> molecule and the  $CH_3^+$  cation, (2) the positive charge is well delocalized at the two fragments even at very large C-N distances. This behavior is definitely artifactual and is typical of the so-called 'inverse symmetry breaking' phenomenon.<sup>[56]</sup> Inverse symmetry breaking is an inherent character of the existing DFT functionals that lead to wrong dissociation limit and the appearance of fictitious TS.<sup>[57]</sup> In the case of the model systems 2 and 3, B3LYP/6-31+G\*\* calculations experienced again an inverse symmetry breaking phenomenon when the C-N bond dissociates. Fictitious TS were then localized on the corresponding PES. HF results, as well as MP2 and CISD ones which include electronic correlation contributions, show no barrier on the energy curves corresponding to the C-N bond dissociation. In fact, we speculate that the direct C-N bond cleavage does not involve any TS, neither for the three model systems nor for the substituted benzylpyridinium cations.

Let us illustrate our argument on the example of system 1,  $CH_3NH_3^+$  (X<sup>1</sup>A<sub>1</sub>). The expected products of the C–N bond cleavage are  $CH_3^+$  and  $NH_3$ . These two species can also be bound through an hydrogen-bond involving a polarized  $^+C-H$  bond and the N atom. The structure of the hydrogen-bond complex,  $CH_3^+\cdots NH_3$  (X<sup>1</sup>A'), is displayed with that of  $CH_3NH_3^+$  species in Fig. 5. The *ab initio* calculations reveal that, when the C–N bond dissociation proceeds, the PES crosses the PES corresponding to the dissociation of  $CH_3^+\cdots NH_3$  complex. This is shown in Fig. 6: at the CISD/6-31+G\*\* level of theory, the two surfaces cross at



Scheme 2. Model systems.



**Figure 4.** Potential energy curve, computed at different levels of theory, corresponding to the C–N bond dissociation of model system 1 depicted in Scheme 2.



Figure 5. Structures of  $CH_3NH_3^+$   $(C_{3\nu})$  and  $CH_3^+\cdots NH_3$   $(C_s)$  species represented with some symmetry elements.

 $d_{C-N} = 3.4$  Å and the energy is well below the asymptote. It is an avoided crossing (going from  $C_{3v}$  to  $C_s$  symmetry, the  ${}^{1}A_{1}$ electronic state of  $CH_{3}NH_{3}^{+}$  reduced to  ${}^{1}A'$ , which correlates with the electronic state of  $CH_{3}^{+}\cdots NH_{3}$ ), meaning that the C–N bond cleavage will shift to an hydrogen-bond dissociation mechanism. Hence,  $CH_{3}^{+}$  and  $NH_{3}$  species could be formed from an initial C–N bond cleavage without encountering any potential energy barrier. The same dissociation mechanism was found for model systems 2 and 3 as illustrated in Fig. 6. This should remain valid for the substituted benzylpyridinium cations; hence, the direct C–N bond cleavage does not involve any TS.

#### C–N bond dissociation energies

As no barrier exists on the PES corresponding to the direct C–N bond cleavage of the substituted benzylpyridinium ions, the critical energy  $E_0$  for this mechanism will be to assimilate the heat of reaction computed at T = 0 K. The question arising is: which computational method(s) should be retained to get accurate values? Fig. 7 displays for 11 benzylpyridinium cations the Gibbs energy of dissociation computed at different levels of theory. For several cations, the appearance energies (AE) corresponding to C–N fragment ions appearance have been obtained by Katritzky *et al.*<sup>[58]</sup> and are also presented for comparison purpose.

Going from the *N*-(4-methoxybenzyl)pyridinium to the *N*-(4-nitrobenzyl)pyridinium, all calculations agree to predict the same trend. Nevertheless, some differences appear if we look individually at each substituted benzylpyridinium ions: the computed Gibbs energies of reaction can differ by more than 65 kJ mol<sup>-1</sup>. B3LYP/6-31+G<sup>\*\*</sup> calculations yield systematically the lowest values while the greatest ones are obtained at the MP2/6-31+G<sup>\*\*</sup> level of theory. Focusing on the non-substituted benzylpyridinium, the *N*-(4-methoxybenzyl)pyridinium, the *N*-(4-methylbenzyl)pyridinium and the *N*-(4-nitrobenzyl)pyridinium cations, the comparison between the AE values and the computed Gibbs energies clearly



**Figure 6.** Potential energy curves, computed at the CISD/6-31+G<sup>\*\*</sup> level of theory, corresponding to the dissociation of model systems 1, 2 and 3. For each system, the C–N bond dissociation is represented by a continuous line and the hydrogen bond (N···H) dissociation is represented by a dashed line.



**Figure 7.** Gibbs energy of dissociation (kJ mol<sup>-1</sup>), computed at different levels of theory, of various substituted benzylpyridinium species. Available appearance energies (AE) corresponding to C–N fragment ions appearance are reported with uncertainty range. AE values are issued from collisionally activated dissociation experiments using FTICR mass spectrometry. The substituted benzylpyridinium species were prepared with an internal energy corresponding to a nominal temperature of 350 K.<sup>[58]</sup>

shows that G3(MP2)//B3LYP and QCISD//MP2 methodologies are the best performers: the average unsigned deviations from experiment are, respectively, 3.6% and 3.8%. The two others AE values reported for the N-(4-bromobenzyl) pyridinium and the N-(4chlorobenzyl)pyridinium differ severely from the predicted Gibbs energies. On one hand, experimental values are far below the average computed values (the agreement with B3LYP/6-31+G\*\* results seems fortuitous). On the other hand, all the calculations agree to predict a greater Gibbs energy of dissociation for the N-(4bromobenzyl)pyridinium and the N-(4-chlorobenzyl)pyridinium than for the N-(4-methylbenzyl)pyridinium, whereas the AE values do not follow at all this trend. The best sounding explanation for this discrepancy is that the measured AE values correspond to another reaction than the one depicted in Scheme 1, the C-N bond cleavage of the N-(4-bromobenzyl)pyridinium and the N-(4chlorobenzyl)pyridinium could notably yield tropylium cations (in conjunction with the formation of pyridine) as evidenced for other substituted benzylpyridinium ions.[42]

#### Evaluation of the internal energy distribution of the electrosprayed ions

As illustrated in Fig. 1, the experimental SY method including the kinetic shift uses the appearance threshold energy values for



**Figure 8.** Experimental survival yields of the seven different substituted benzylpyridinium cations plotted as a function of the different threshold energy values calculated by considering either no initial kinetic energy for the ions emitted in the ESI interface (open circle) or a supersonic jet sampled by the skimmer (black squares). Note that the experimental data have been obtained with a 120-V cone voltage and a 150 °C ESI source temperature.

evaluating  $P(E_{int})$ . The  $E_{thr}$  parameter can be defined as the energy at which the rate constant for the dissociation  $k(E_{int})$  is equal to  $1/\tau$ , where  $\tau$  is the residence time of the ion in the ESI interface before entering the acceleration zone. The residence time of each benzylpyridinium cation determines the time scale of the experiment required to observe their fragmentation. Depending on whether the ions are sampled by the skimmer aperture downstream or upstream the Mach disc, the velocity v in the interface will be expressed using either Eqn (6) or (7) in which an additional term related to a supersonic velocity value is considered:

$$v = \sqrt{\frac{2}{mu}}\sqrt{e \cdot \Delta V} \tag{6}$$

$$v_{\text{Mach}} = \sqrt{\frac{2}{mu}} \left( \sqrt{\text{KE}_{\text{initial}} + (e \cdot \Delta V)} \right)$$
(7)

In Eqns (6) and (7), the common parameters are the atomic mass unit u, the elementary charge e, the potential difference  $\Delta V$ between orifices 1 and 2 (Fig. 3) and the mass of the ion, m. In Eqn (7), KE<sub>initial</sub> is the initial kinetic of ions sampled in the expansion jet by considering there velocity value close to Mach 1, i.e. 343 m s<sup>-1</sup>. The obtained  $\tau$  values correspond to the sum of the residence time of an ion formed at OR1, accelerated under a potential difference corresponding to the CV value and the time of flight in the ion guide (Fig. 3). The residence time of the substituted benzylpyridinium cations has been calculated by taking into account the mass of the seven studied species. From Eqn (6) (skimmer-tip outside the Mach disc), the mean value is of 27.25 us with a 1.039 standard deviation. From Eqn (7) (ions sampled in the silence zone), the mean value is of 0.1425 µs with a 0.0054 standard deviation. The approximate rate constant necessary to produce fragments observed on the ion source mass spectrum is equivalent to the reciprocal of the residence time of ions. As a result, the two residence time values have been considered for the estimation of the different  $E_{thr}$  from the RRKM curves  $\log[k(E_{int})]$  versus  $E_{int}$ .

The experimental SYs of the seven substituted benzylpyridinium cations recorded at a 120 V orifice 1 voltage value are plotted as

a function of the appearance threshold energies (Fig. 8). This voltage value has been selected with regard to the SYs of the *N*-(4-methoxybenzyl)pyridinium and the *N*-(4-nitrobenzylpyridinium) of  $6.4 \times 10^{-3}$  and 0.99, respectively. These experimental data avoid to add manually any point at the 0 and 1 ordinates. This confirms that the SY measurements fully characterize the internal energy distribution of the ESI source used for this study. The experimental SY of each benzylpyridinium cation has been plotted as a function of the *E*<sub>th</sub> values. These ones are calculated from the two considered kinetic shifts, i.e. skimmer-tip downstream or upstream the Mach disc. The obtained SY(*E*<sub>int</sub>) data of Fig. 8 are then fitted with single, symmetrical sigmoids (Eqn 8):

$$y = \frac{a}{e^{-(x-x_0)/b} + 1}$$
 (8)

With these experimental data, we did not need to use the procedure involving a fit refined by the use of two sigmoids as described by Gabelica et al. in the case of the P(Eint) determination in a heated capillary nano-electrospray source.<sup>[30]</sup> The position of the first point relative to the SY of the most fragile N-(4methoxybenzyl)pyridinium cation is close to 0. This observation confirms that under the experimental conditions of the present study, this ion population is entirely desolvated and has enough energy to completely fragment. The position of the last point that characterizes an undissociated N-(4-nitrobenzyl)pyridinium ion allows to envisage a symmetric distribution of the internal energy. The curves obtained with the fitting procedure are shown in Fig. 8. The best fit is obtained for a = 1 in Eqn (8) and with the Ethr values calculated from molecular parameters obtained at the QCISD//MP2 level of theory. If  $a \neq 1$  in Eqn (8), the sigmoid does not fit the data for SY(E<sub>int</sub>) values close to the unity. In addition, with the QCISD//MP2 calculation, the fit is obtained with a standard deviation  $\geq$  0.99, whereas with the E<sub>thr</sub> values obtained from the G3(MP2)//B3LYP methodology, the standard deviation does not exceed 0.84. The curves reported in Fig.8 show that whatever the selected kinetic shift, the experimental SY values related to the electrosprayed cations are well localized on the sigmoid parts that will determine either the shape and the width of the resulting  $P(E_{int})$ . The results of this single sigmoid fitting procedure are represented in Fig. 8 only in the case of the QCISD//MP2 methodology.

The derivates of the calculated sigmoids are expressed as follows: the b and  $x_0$  parameters being extracted from the fit procedure using Eqn (9):

$$\frac{dy}{dx} = \frac{e^{-(x-x_0)/b}}{b(e^{-(x-x_0)/b}+1)^2}$$
(9)

The internal energy distribution then obtained is reported in Fig. 9. From the depicted curves, it appears that the SY method including the kinetic shift allows to evaluate two different internal energy distributions as a function of the assumed skimmer tip position. For ions sampled outside the Mach disc, where the density of gas is high and the ion velocity is low, the mean internal energy should be dramatically lower than the  $P(E_{int})$  of the benzylpyridinium ions sampled in a supersonic jet. In the first case, this  $P(E_{int})$  is characterized by a maximum at 5.385 eV, whereas in the second case,  $P(E_{int})$  is centered around the 7.544 eV value. Nevertheless, in both cases, the full widths at half maximum (FWHM) are of low values (Fig. 9). The internal energy distribution of the ions emitted in an ESI source interfaced with a sector



**Figure 9.** Internal energy distribution derivate from the curves of Fig. 8 using Eqn (10) and by considering: ( $\bullet \bullet \bullet$ ) no initial kinetic energy of the ions and (- - -) a supersonic velocity for the initial kinetic energy of the ions. The centroid (§) and the full width half maximum (\*) of each distribution are noted directly above the corresponding curves.

mass spectrometer is thus very narrow in comparison with the  $P(E_{int})$  reported in the literature in the case of a quadrupole mass spectrometer.<sup>[26]</sup> For the latter case, a maximum of the  $P(E_{int})$ curve at  $\sim$ 5 eV is associated with a FWHM of the distribution of  $\sim$ 6 eV. Such a result obtained with the experimental SY method is in agreement with a thermal-like distribution for ions at 1480 K. In order to confirm one of the two  $P(E_{int})$  reported in Fig. 9, the MassKinetics software was used. The aim was to obtain a  $P(E_{int})$ similar for all the cations when the theoretical SY calculated by MassKinetics is equal to the experimental SY. In this case, the choice of a same temperature parameter for describing the initial internal energy does not allow to fit the theoretical SY and the experimental SY. The internal energy distributions that allow to calculate, for each of the seven cations, a theoretical SY equal to the experimental SY, are not thermal but dramatically narrower. The internal energy distributions of the substituted benzylpyridinium cations considered as a fitting parameter in MassKinetics are reported in Fig. 10.

Each curve corresponds to the  $P(E_{int})$  of ions produced in the gas phase and sampled by the orifice 2 after their activation in the declusterization area (Fig. 3). The FWHM of the curves  $P(E_{int})$  versus E<sub>int</sub> is 0.47 eV for all the substituted benzylpyridinium cations with a maximum of  $P(E_{int})$  that can be evaluated at 5.15 eV  $\pm$  3% as a function of the studied specie (Fig. 10(a)). For each cation, the theoretical SY values fit with the experimental data as illustrated by Fig. 10(b) where a linear regression is obtained with a standard deviation R = 0.999. The internal energy distributions calculated by MassKinetics (Fig. 10) correspond to  $P(E_{int})$  obtained by the way of the experimental SY method including the kinetic shift (Fig. 9) when the ions are sampled outside the Mach disc. One can remark that the values of the maximum of the curves and the FWHM are similar. Such results confirm first that the ions electrosprayed in the sector mass spectrometer used for this study are characterized by a very narrow internal energy distribution. This one cannot be compared in a first approximation to a thermal distribution. Then, this comparison shows that the two used SY methods are relevant for such a study if the kinetic shift and the theoretical parameters are properly chosen. This kinetic shift corresponds to ions sampled upstream the Mach disc. At least, the linear regression depicted in Fig. 10(b) suggests that only one thermometer ion can be chosen



**Figure 10.** (a) Internal energy distributions calculated by MassKinetics for  $(- \bullet \bullet -)$  the *N*-(4-methoxybenzyl)pyridinium ion  $(p-CH_3O)$ , (----) the *N*-(4-methylbenzyl)pyridinium ion  $(p-CH_3)$ ,  $(\bullet \bullet \bullet)$  the *N*-(4-fluorobenzyl)pyridinium ion (p-F), (-----) the *N*-(benzyl)pyridinium ion (H), (----) the *N*-(3-methylbenzyl)pyridinium ion  $(m-CH_3O)$ , (----) the *N*-(3-methoxybenzyl)pyridinium ion  $(m-CH_3O)$  and  $(\bullet \bullet \bullet)$  the *N*-(a-methoxybenzyl)pyridinium ion  $(m-CH_3O)$  and  $(\bullet \bullet \bullet)$  the *N*-(benzyl)pyridinium ion  $(p-NO_2)$ . (b) Linear regression calculated from the plot of the theoretical SY *versus* the experimental SY by using the internal energy distributions depicted in (a) for the calculation of the theoretical SY.

for describing the internal energy of the electrosprayed ions. This is in agreement with one of the assumptions mentioned in the case of the use of the experimental SY method (see Section on Methods).

As the observed internal energy distribution cannot be compared with a 'thermal-like' Boltzmann distribution, it could be interesting to verify if an increase in the potential difference in the declustering area leads to a warm-up of ions as often described in the literature for other ESI sources.<sup>[25,26]</sup> With MassKinetics, the internal energy distribution of the electrosprayed ions has been evaluated at different OR1 voltage values (Fig. 3). For each voltage, one substituted benzylpyridinium cation could be used as thermometer ion with regard to the linear regression of Fig. 10(b) that allows to consider at least this ion as representative of the overall population. In our case, we have chosen to consider two ions in order to compare the evolution of the  $P(E_{int})$ with the variation of the source acting parameters such as the temperature and the voltage value. Among the selected ions, the benzylpyridinium and the N-(3-methylbenzyl)pyridinium cations are the common thermometer ions for measurements performed at three different temperatures by keeping the voltage value constant, respectively, at 90 and 120 V. The evaluated internal energy distributions that allow to obtain the same experimental and theoretical SY in MassKinetics are illustrated in Fig. 11. The comparison of the SY values obtained for the two species selected among the seven substituted benzylpyridinium cations is reported in Table 2. From the  $P(E_{int})$  curves of Fig. 11, it appears that an increase in the potential difference between OR1 and OR2 of about 30 V leads to a shift of  $P(E_{int})$  to the high-energy values rather than a broadening of the curves. Indeed, for a source temperature of 423 K, the increase in the voltage from 90 to 120 V leads to a shift of the maximum of the curves from 4.4 to 5.25 eV, respectively. By contrast, for a same voltage value, e.g. 90 V, the change in temperature, i.e. from 423 to 523 K, leads to an increase in the FWHM value from 0.41 to 0.83 eV, whereas the maximum of the curves is only shifted of 0.4 eV. With regard to the broader  $P(E_{int})$  that has been evaluated in Fig. 11, it must be



Figure 11. Internal energy distribution calculated by MassKinetics as a function of the source temperature and the potential difference in the declusterization area (\*90 V and #120 V). Note that the value in brackets indicates the temperature of orifice 1.

<b>Table 2.</b> Comparison of the experimental and the theoretical survival yields obtained for two substituted benzylpyridinium as the cone voltage value and the temperatures of the ESI interface (see Experimental Section)							
Source temperature (orifice 1 temperature)	Experimental SY		Theoretical SY		Orifice 1 voltage (V)		
423 K (353 K)	0.894 (p-CH <sub>3</sub> )	0.96 (H)	0.878 (p-CH <sub>3</sub> )	0.96 (H)	90		
	0.325 (H)	0.775 ( <i>m</i> -CH <sub>3</sub> )	0.325 (H)	0.74 ( <i>m</i> -CH <sub>3</sub> )	120		
473 K (361 K)	0.797 (p-CH <sub>3</sub> )	0.906 (H)	0.75 (p-CH <sub>3</sub> )	0.91 (H)	90		
	0.248 (H)	0.367 ( <i>m</i> -CH <sub>3</sub> )	0.246 (H)	0.377 ( <i>m</i> -CH <sub>3</sub> )	120		
523 K (371 K)	0.485 (p-CH <sub>3</sub> )	0.742 (H)	0.479 (p-CH <sub>3</sub> )	0.436 (H)	90		
	0.085 ( <i>m</i> -CH <sub>3</sub> )	0.128 (m-CH <sub>3</sub> O)	0.045 (m-CH <sub>3</sub> )	0.127 ( <i>m</i> -CH <sub>3</sub> O)	120		

mentioned that for a 523-K temperature and an orifice 1 voltage value of 120 V, a FWHM value of only  $\sim$ 1 eV is observed. Such narrow internal energy distributions have also been disclosed in Fig. 9. The increase in the voltage in the declustering area cannot be assimilated in a first approximation to a warm-up of the ion population in all the vibration modes.

# Conclusions

The approach that consists in combining the experimental SY method of De Pauw *et al.* and the theoretical SY method using MassKinetics software of Drahos and Vékey allows to evaluate two comparable internal energy distributions of substituted benzylpyridinium cations electrosprayed in an ESI source interfaced with a sector mass analyzer. This is particularly relevant when quantum chemical calculations are performed at the QCISD/6- $31+G^{**}//MP2/6-31+G^{**}$  level of theory for the selected thermometer ions. The methods used for evaluating  $P(E_{int})$  in the JEOL ESI source disclose that the internal energy distribution is

not comparable to a thermal distribution for an ESI source of a quadrupole mass spectrometer.<sup>[25-27]</sup> The shape of the internal energy distribution is narrower than a thermal distribution usually calculated from the vibrational state density of the thermometer ions.<sup>[24]</sup> The interpretation of such a behavior is not trivial. It can be regarded either through the acting of a source having no curtain gas incoming in the first pumping stage or through the characteristics of the analyzer used for the study. In the case of the JEOL ESI device, the disclosed non-thermal internal energy distribution might be related to ions sampled by the skimmer downstream the Mach disc, i.e. into the axis of the expansion jet. Indeed, the selected population should have frozen translational, rotational and vibrational temperatures with a relaxation process of low efficiency for some vibration modes.<sup>[59,60]</sup> However, this is not the case, because the ions should have an initial velocity close to the Mach number with an internal energy distribution in agreement with a short resident time of ions in the source. The comparison of the two  $P(E_{int})$  obtained shows in fact that the distributions are comparable only for an ion sampling outside the Mach disc, i.e. as the randomized trajectory of ions is taken into account. By reporting to the comments of the introduction section, the probing of such ions downstream the Mach disk should lead rather to a thermal-like internal energy distribution as previously described for the ESI source of the PE SCIEX API 165 guadrupole mass spectrometer.<sup>[27]</sup> The JMS 700 ESI source used for this study presents the same characteristics as the PE SCIEX. By focusing onto the differences in the analyzer used for these studies, one can report the works of Anderson et al.<sup>[61]</sup> They have shown that in a quadrupole mass spectrometer acting with an ESI source, the direct current field between the quadrupole rods is responsible to a radially directed acceleration of the parent ions. The broadening in kinetic energy is then converted in a broadening in internal energy by collisions with the neutrals due to the jet formation in the expansion area of the ESI source. In the case of the SY method, some substituted benzylpyridinium cations should be lost into the analyzer as parent ions, whereas the benzylium ions produced in the source are more likely to survive in the quadrupole. Such an interpretation involving the analyzer rather than the source can find a confirmation through the results obtained by Gabelica et al.<sup>[30]</sup> In this paper devoted to the internal energy distribution of benzylpyridinium ions emitted from a nano-electrospray source interfaced with TOF analyzer, distributions remain narrow whatever the gas pressure in the first pumping stages, the voltage and temperature conditions. The conclusions of the authors concerning the fact that 'low temperature-high acceleration voltage' conditions are not equivalent to 'high temperature-low acceleration voltages' can be applied to the results shown in Fig. 11, in the case of the sector mass spectrometer.

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