

B3LYP DFT molecular orbital approach, an efficient method to evaluate the thermochemical properties of MALDI matrices

Sophie Bourcier, Yannik Hoppilliard*

DCMR, UMR CNRS 7651, Ecole Polytechnique, 91128 Palaiseau Cedex, France

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Abstract

The thermochemical properties of seven UV-MALDI matrices and two IR-MALDI matrices were evaluated by means of *ab initio* molecular orbital calculations using the B3LYP functional density approach with 6-31+G(d,p) and 6-311+G(2d,2p) basis sets. These thermochemical properties include ionization energy, proton and electron affinities of neutral molecules and proton affinity of deprotonated molecules. The chosen UV-MALDI matrices are: 2,5-dihydroxybenzoic acid (2,5DHB), nicotinic acid (NA) and 2-aminonicotinic acid (2ANA), picolinic acid (PA) and 3-hydroxypicolinic acid (3HPA), 2-aminobenzoic acid (2ABA) and 4-nitroaniline (4NiAn) while the studied IR-MALDI matrices are urea (U) and glycerol (G). The proton affinities of neutral and deprotonated molecules are in very good agreement with the available experimental values (the differences between experimental and calculated values are smaller than the precision on the experimental measurements); the calculated ionization energies are close to the experimental values of adiabatic electronic transitions; the calculated electron affinities are new and cannot be compared with corresponding experimental values since they are not available. (Int J Mass Spectrom 217 (2002) 231–244) © 2002 Elsevier Science B.V. All rights reserved.

Keywords: MALDI matrices; Proton affinity; Ionization energy; Electron affinity; DFT molecular orbital calculations; Mass spectrometry

1. Introduction

The invention of matrix-assisted laser desorption–ionization (MALDI) has resulted in a major extension in the size of molecules that can be analyzed by mass spectrometry [1]. Although this desorption–ionization technique is successfully applied to the analysis of biological, biochemical and industrial samples, the desorption–ionization mechanisms of molecules remains not fully understood. Although it does not certainly reflect the reality, both desorption and ionization mechanisms for MALDI mass spectrometry use to be considered separately, with the aim of

simplifying the theoretical approach. The laser fluence used in MALDI experiments leads to the formation and the expansion of a plume. The energy required for the desorption step is not well established but certainly depends on the sublimation energy of the solid sample, greatly depending upon the polar functional groups substituting the molecules and upon their relative disposition in the space which define the strength of interactions between molecules in the condensed phase and during the desorption–ionization process. Models for ion formation in MALDI mass spectrometry were summarized in a recent and excellent review by Zenobi and Knochenmuss [2] and discussed again more recently by these authors [3] and by Karas et al. [4]. Most of ion formation models take into account the

* Corresponding author. E-mail: yannik@dcmr.polytechnique.fr

thermochemical properties of matrices. However, the selection of optimal matrices continues to be based upon empirical methods since the thermochemical properties remain unavailable for most of the matrices and experimentally difficult to obtain.

In this work, we propose an alternative for the experimental determination of thermochemical properties of molecules and ions: their evaluation by *ab initio* molecular orbital calculations. These thermochemical properties include: ionization energy, proton and electron affinities of neutral molecules and proton affinity of deprotonated molecules. Recently, we showed [5] that *ab initio* molecular orbital calculations using the B3LYP functional density approach

at the B3LYP/6-311+G(2d,2p) level of theory give a very good calculated evaluation of thermochemical properties of DHB. This is why, in the present work, we have also used this level of theory to evaluate and compare the thermochemical properties of nine compounds, well known as efficient MALDI matrices: seven aromatic compounds used for UV-MALDI experiments which are: 2,5-dihydroxybenzoic acid (2,5DHB), nicotinic acid (NA), 2-aminonicotinic acid (2ANA), picolinic acid (PA), 3-hydroxypicolinic acid (3HPA), 2-aminobenzoic acid (2ABA) and 4-nitroaniline (4NiAn) and two matrices more specific of IR-MALDI experiments which are urea (U) and glycerol (G) (Fig. 1). For each of these compounds

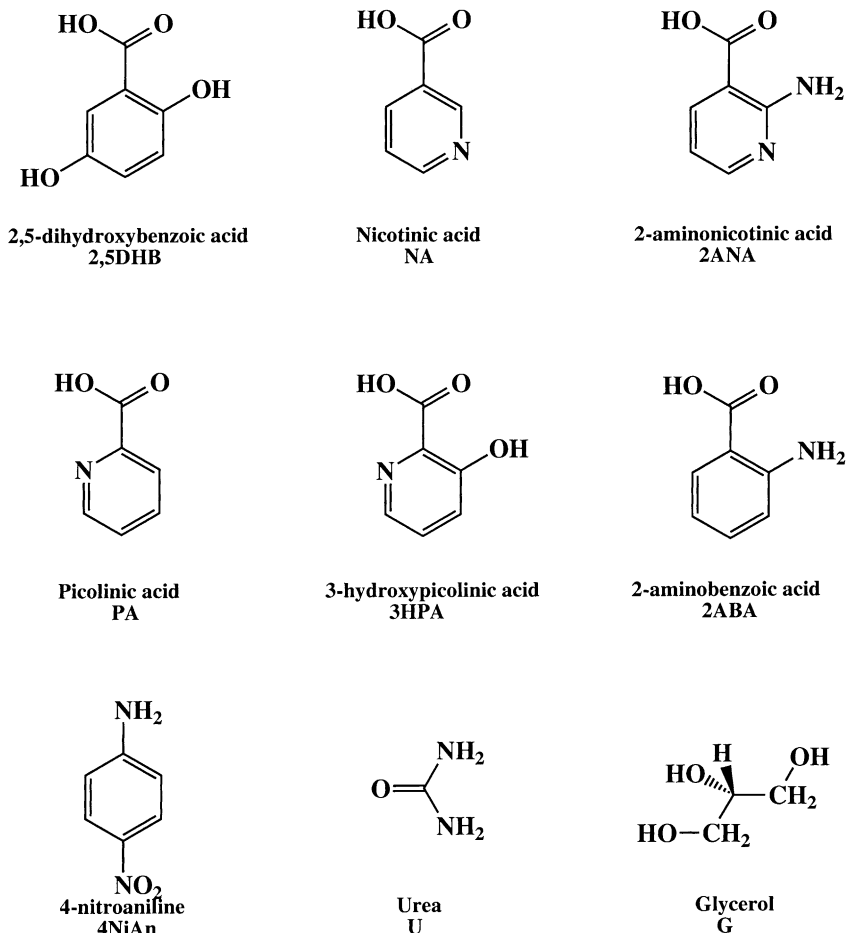


Fig. 1. Chemical structures of studied molecules.

we have searched for the most stable structure describing the neutral, protonated and deprotonated molecules and the positive and negative molecular radical ions. The calculated thermochemical properties are deduced from the differences in energies between the most stable neutral and ionized forms of each molecule.

2. Theoretical data

The best accuracy between calculated and experimental basicities and acidities, in the gas phase, may be obtained by Gaussian 2 procedures (G2 [6], G2(MP2) [7] and G2(MP2,SVP) [8] as shown for small molecules (less than four heavy atoms) by Smith and Radom [8,9] and Merrill and Kass [10] and for small neutral α -aminoacids by Yu et al. [11] and by Topol et al. [12]. However, for molecules with several heavy atoms, these procedures require considerable computational times. That is why some of these authors [9,10,12] have compared the results obtained with G2 procedures with those calculated using various density functional theory (DFT) methods. They conclude that gas phase acidity values calculated by using different levels of DFT are highly consistent with experimental values and of comparable accuracy to those computed from high-level (G2) *ab initio* calculations but at a significantly lower computational cost.

Among the DFT methods, the most popular is the B3LYP hybrid method [13] which was shown to give good results for deprotonation energies with both 6-311+G** and 6-311+G(3df,2p) basis sets [13]. Catalan et al. [14] have investigated gas-phase acidities and basicities of azoles at the B3LYP/6-311+G(3df,3dp) level of theory and found excellent correlation between the experiment and calculations. The gas-phase acidities and basicities for 49 acids and 32 bases, calculated using B3LYP hybrid DFT method and 6-31G*, 6-31+G*, 6-311+G** and 6-311+G(3df,3dp) basis sets were compared with corresponding experimental values [15]. The authors conclude that B3LYP/6-311+G** level of

theory seems to be the best bargain between accuracy and speed of calculations for acidities and basicities whereas the average absolute error of calculated acidities and basicities (≤ 12 kJ/mol) is comparable to experimental uncertainty.

In the other hand, the well-established ionization energies and electron affinities of 146 molecules were evaluated using G2 and DFT theories [16]. It is found that G2 theory is the most reliable method. However, the B3LYP functional performs the best of the seven DFT methods investigated.

In this work, taking into account the earlier results of the literature, basicities, acidities, ionization energies and electron affinities of MALDI matrices are calculated using the B3LYP hybrid DFT method with 6-31+G** and 6-311+G(2d,2p) basis sets. All calculations were carried out using the Gaussian 94 [17] and Gaussian 98 [18] program packages. Geometries were optimized without any symmetry constraint. Geometry optimizations and vibrational frequency calculations were carried out at the B3LYP/6-31+G**=B3LYP/6-31+G(d,p) level. Final energetics were obtained with B3LYP/6-311+G(2d,2p) functional on the optimized geometries.

The proton affinity (PA) of neutral (M) and deprotonated ($[M-H]^-$) molecules were calculated as enthalpy changes at 298 K in reactions (1) and (2), respectively



PA can be obtained as

$$PA_{298} = \Delta E_{elec} + \Delta(PV) + \Delta(ZPE) + \Delta E_{vib(298)} + \Delta E_{rot(298)} + \Delta E_{trans(298)} \quad (3)$$

$$PA_{298} = \Delta E_{elec} + 298R + \Delta E_{therm(298)} + \Delta E_{rot(298)} + \Delta E_{trans(298)} \quad (4)$$

where ΔE_{elec} is the variation in internal energy that arises directly from computations at the B3LYP/6-311+G(2d,2p) level; 298 K (2.5 kJ/mol) represents the $\Delta(PV)$ term necessary to convert

internal energy in enthalpy (in the ideal gases approximation); $\Delta E_{\text{therm}(298)}$ is the variation in thermal energy at 298 K, including the variation in zero-point vibration energies $\Delta(\text{ZPE})$ and the vibrational enthalpy correction ($\Delta E_{\text{vib}(298)}$) derived by computed harmonic frequencies at the B3LYP/6-31+G(d,p) level. The corrections due to translation and rotation ($\Delta E_{\text{rot}(298)} + \Delta E_{\text{trans}(298)}$) can be treated classically, using the equipartition theorem. Thus, considering that in the final state, we have two species present and one in the initial state, and also that the proton has only translational degrees of freedom, we may write: $\Delta E_{\text{trans}(298)} = 3/2RT$ (3.7 kJ/mol) and $\Delta E_{\text{rot}(298)} = 0$.

The ionization energy (IE) and the electron affinity (EA) of neutral molecules were calculated as enthalpy changes in reactions (5) and (6), respectively



IE or/and EA can be obtained as

$$\begin{aligned} EI_{298} \text{ or/and } EA_{298} \\ = \Delta E_{\text{elec}} + \Delta(\text{PV}) + \Delta(\text{ZPE}) + \Delta E_{\text{vib}(298)} \\ + \Delta E_{\text{rot}(298)} + \Delta E_{\text{trans}(298)} \end{aligned} \quad (7)$$

In our calculations the following approximated expression was used:

$$EI_{298} \text{ or/and } EA_{298} = \Delta E_{\text{elec}} + \Delta E_{\text{therm}(298)} \quad (8)$$

where ΔE_{elec} is the variation in internal energy at the B3LYP/6-311+G(2d,2p) level; $\Delta(\text{PV}) = 0$; $\Delta E_{\text{therm}(298)}$, the variation in thermal energy at 298 K is derived by computed harmonic frequencies at the B3LYP/6-31+G(d,p) level and includes $\Delta(\text{ZPE})$, $\Delta E_{\text{vib}(298)}$, $\Delta E_{\text{rot}(298)}$ and $\Delta E_{\text{trans}(298)}$. In both cases, the neutral and ionic species are calculated in their ground state and consequently the calculated thermochemical data correspond to adiabatic transitions.

3. Results and discussion

The performance of 2,5-dihydroxybenzoic acid as a matrix for laser desorption–ionization mass spectrometry of proteins was described by Hillenkamp and coworkers [19,20]. Nicotinic acid was one of the first aromatic compound tested as UV matrix for molecular weight determination of large molecules by LDI [21,22]. This matrix was also demonstrated to be well suited to IR-MALDI analysis of large proteins [23]. 2-Aminonicotinic acid and 2-aminobenzoic acid appear in a study where a large number of polar aromatic molecules were investigated as UV matrices [24]. Readily reproducible LDI mass spectra of underivatized mixed-base single-stranded DNA oligomers ranging from 10 to 67 bases have been generated using, for the first time, 3-hydroxy-picolinic acid as UV matrix [25]. Then Tang et al. [26] showed picolinic acid superior to 3-hydroxy-picolinic acid as UV matrix for MALDI of oligonucleotides. 4-Nitroaniline is included in the substituted benzene derivatives containing basic amino groups screened as UV-matrices for the MALDI analysis of proteins and oligonucleotides [27]. Urea and glycerol were tested for the first time as IR-matrices by Overberg et al. [23] in 1990. Urea is the only matrix which has a physiological pH.

Among the thermochemical properties including PA, gas-phase basicity (GB), ΔH_{acid} , IE, EA, of compounds investigated in this work, some are known experimentally. Experimental proton affinities or gas-phase basicities are available for 2,5DHB [28–32], NA [29,31], PA [31], 3HPA [31], 4NiAn [35] and G [35]. The gas-phase basicities of deprotonated 2,5DHB, NA, 3HPA and 4NiAn were determined using a modified bracketing method [33]. The gas-phase basicity of deprotonated urea is given in the NIST database [35]. For comparison with calculated values, enthalpy values were chosen. Consequently, the experimental values reported in Table 1 are either experimental proton affinities of neutral and deprotonated molecules either proton affinities deduced from experimental gas phase basicities. The PA of a neutral molecule M may be deduced from the gas phase basicity of M by applying the usual entropic correction

Table 1

Calculated and experimental (see text) values of proton affinities (PA) of neutral and deprotonated molecules (kJ/mol), IE and EA of neutral molecules (eV)

Compound	PA(M) calc.	PA(M) exp.	$\Delta H_{\text{acid}}(\text{M})$ = PA[M–H] [–] calc.	$\Delta H_{\text{acid}}(\text{M})$ = PA[M–H] [–] exp.	IE(M) calc.	IE(M) exp.	EA(M) calc.	IE–EA calc.
2,5DHB	852	849–858 ^a	1367	1362 ^b	7.86	8.05 ^c	0.56	7.3
NA	918	900–907 ^d	1400	1399 ^b	9.21	9.38 ^e	0.61	8.6
2ANA	937	–	1398	–	8.15	–	0.47	7.68
2ABA	895	–	1416	–	7.72	–	0.17	7.55
PA	919	907 ^f	1439	–	9.27	–	0.72	8.55
3HPA	903	896 ^f	1378	1397 ^b	8.95	–	1.0	7.95
4NiAn	888	866 ^g	1424	1440–1444 ^b , 1437 ^g	8.29	8.34–8.6 ^h	0.97	7.32
U	875	–	1519	1514 ^g	9.61	9.7 ^e	–0.85	10.45
G	847	875 ^h	1471	–	9.19	–	–0.78	9.97

^aEvaluated from [28–32].

^bEstimated from [33].

^cFrom [34].

^dFrom [29,31].

^eFrom [35] (adiabatic transition).

^fFrom [31].

^gFrom [35].

^hFrom [35]: the lowest IE value corresponds to the adiabatic transition while the highest is the vertical transition.

$\text{PA}(\text{M}) = \text{GB}(\text{M}) - T[\Delta S_{\text{p}}(\text{M}) - S^{\circ}(\text{H}^{+})]$, $\Delta S_{\text{p}}(\text{M})$ is the entropy of protonation of M, $T = 298 \text{ K}$ and $S^{\circ}(\text{H}^{+}) = 109 \text{ kJ/mol}$. $\Delta S_{\text{p}}(\text{M})$ is approximated by the temperature independent expression: $\Delta S_{\text{p}}(\text{M}) = R \ln[\sigma(\text{M})/\sigma(\text{MH}^{+})]$, where R is the universal gas constant while $\sigma(\text{M})$ and $\sigma(\text{MH}^{+})$ are the rotational symmetry numbers of M and MH^{+} . In the hypothesis where the rotational symmetry numbers are the same in M and MH^{+} , $\text{PA}(\text{M})$ is obtained by adding $TS^{\circ}(\text{H}^{+}) = 33 \text{ kJ/mol}$ to the gas phase basicity of M. In the same manner, the PA of anions $[\text{M}–\text{H}]^{-}$ is obtained by adding $TS^{\circ}(\text{H}^{+}) = 33 \text{ kJ/mol}$ to the gas phase basicity of $[\text{M}–\text{H}]^{-}$ [36].

The IE of 2,5DHB was measured recently [34] while those of NA, 4-NiAn and U are known since the 1970s and are tabulated in the NIST database [35]. To our knowledge, the electron affinities of all the studied matrices are unknown.

The calculated thermochemical properties are deduced from the differences in energies between the most stable neutral and ionized forms of each molecule. The different structures of a given species are compared on their total energy: the lowest the energy, the most stable the structure. The structures

of neutral molecules found to be the most stable are given in Fig. 2.

3.1. Proton affinities of neutral molecules

In the studied aromatic compounds, several sites are available for protonation. Taking 2,5DHB as an example, we have investigated 10 structures to describe the protonated 2,5DHB: four structures **A**, **A1**, **A2**, **A3** are different conformations of 2,5DHB protonated on the carbonyl function; three structures, **B**, **C** and **D** correspond to protonation of the hydroxy of the carboxylic function and of the hydroxyl in position 2 and 5 in the phenyl ring, respectively; three structures, **E**, **F** and **G** correspond to protonation on the aromatic ring on carbons 2, 4 and 6, respectively. The various protonated forms are presented in Fig. 3. The total and relative energies of these structures, at the B3LYP/6-31+G** level, are tabulated in Table 2. Among these structures, the most stable one is **A** in which the protonating hydrogen beared by the carbonyl oxygen is stabilized by the oxygen of the hydroxy in position 2. The corresponding PA calculated at the best level of theory following reaction (5) is 852 kJ/mol in excellent

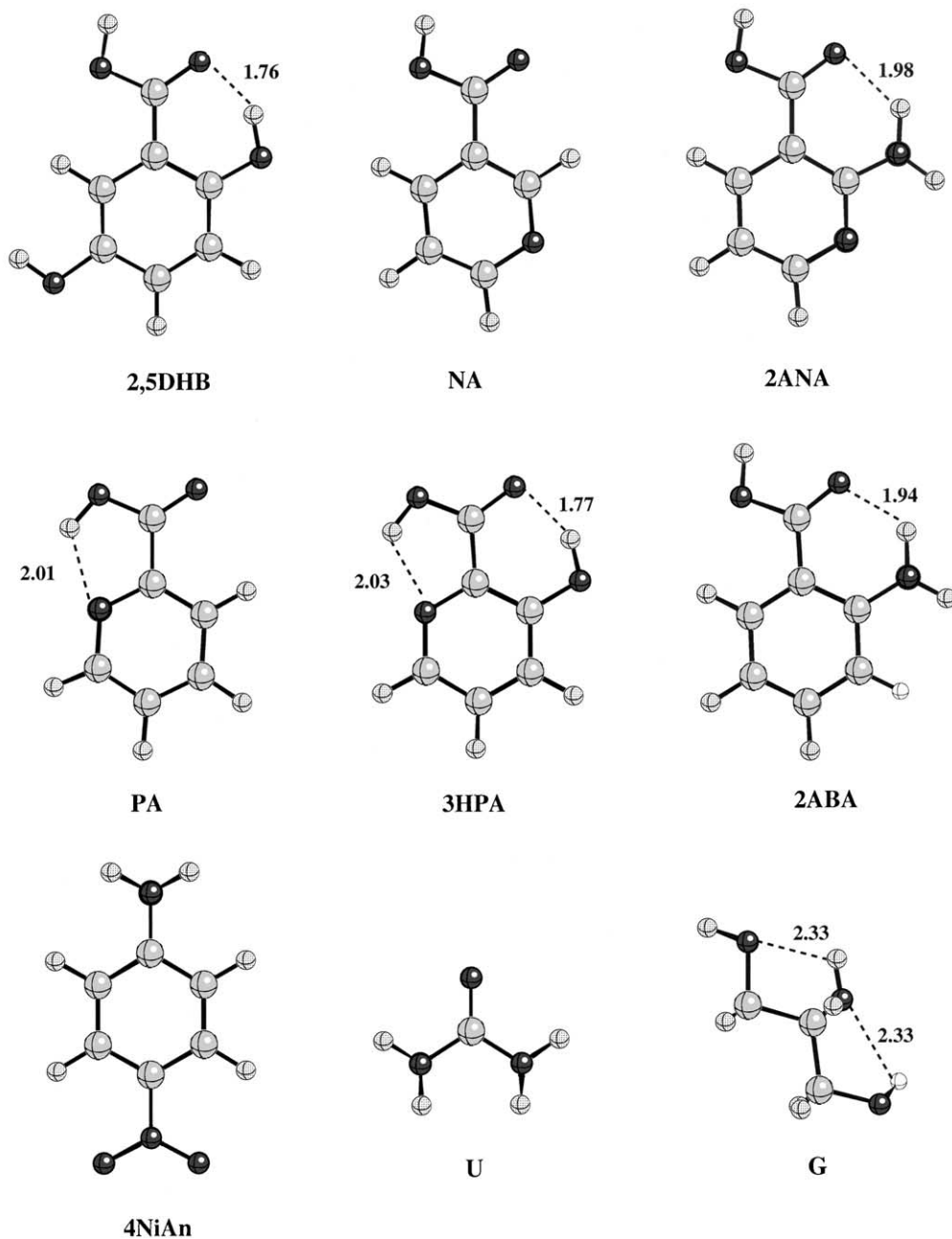


Fig. 2. Optimized geometries (B3LYP/6-31+G** level) of the most stable structures of neutral matrices.

agreement with experimental values comprise between 849 and 858 kJ/mol [28–32].

Since the aromatic ring and the hydroxy groups of 2,5DHB are calculated to be less basic than the

carbonyl function (*vide supra*), for other UV-MALDI matrices (NA, 2ANA, 2ABA, PA and 3HPA) the basicity of CO was only compared with that of the nitrogen atoms. At the B3LYP/6-31+G** level of theory,

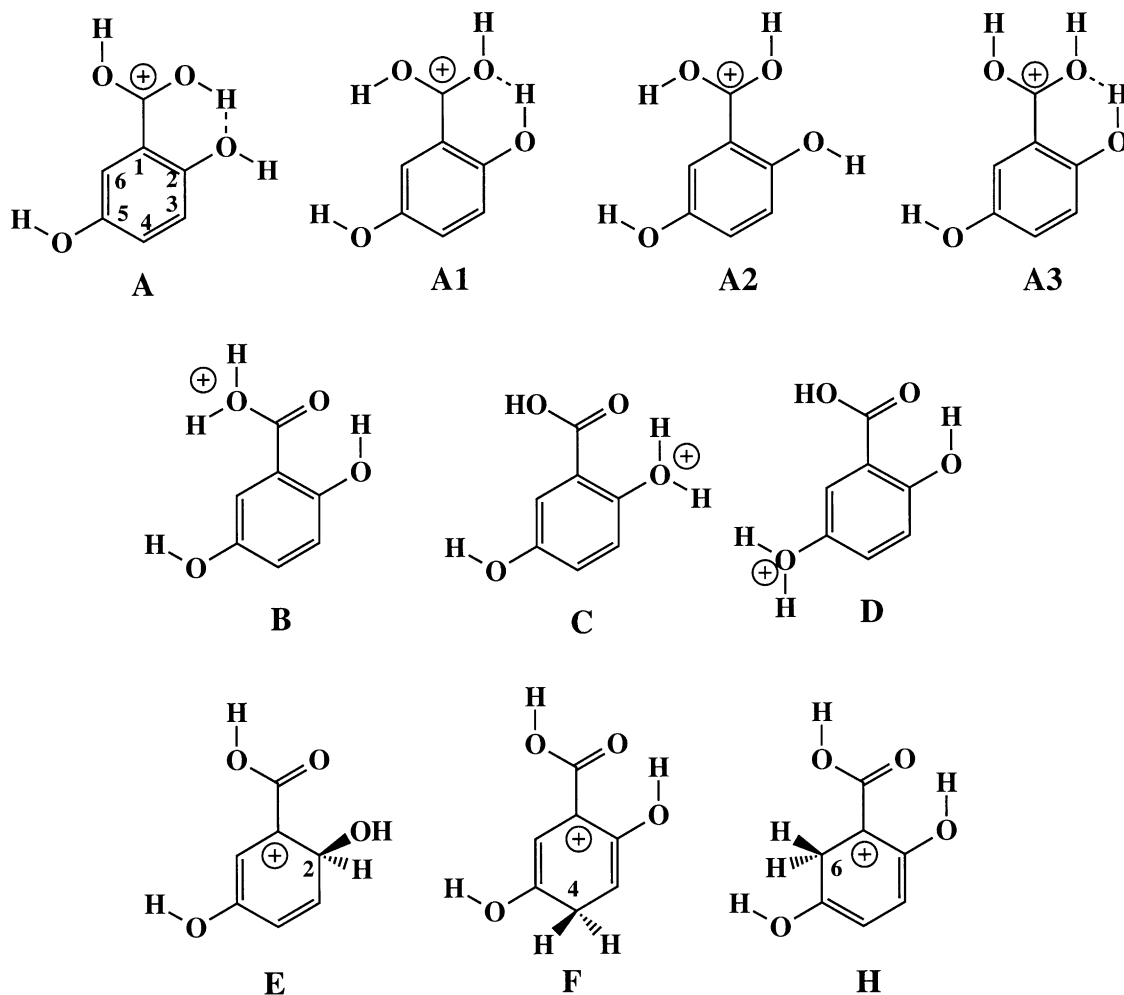


Fig. 3. Protonated structures of 2,5DHB.

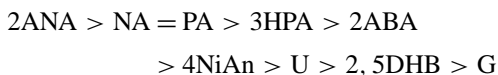
Table 2

Total E_{elec} (hartree) and relative ΔE (kJ/mol) energies of various protonated forms of 2,5DHB

[DHBH] ⁺ ions	E_{elec} , B3LYP/6-31+G** (6-31G**=6-31+G(d,p))	ΔE
A	-571.648688	0
A1	-571.631460	45
A2	-571.631348	46
A3	-571.626237	59
B	-571.613690	92
C	Rearranges into A	-
E	-571.602294	122
F	-571.617837	81
H	-571.627992	54

the nitrogen atoms included in the aromatic ring are shown to be more basic than the amines functions substituting the aromatic ring which are in turn more basic than the carbonyl function of the carboxylic groups. For an example, in the case of 2ANA, the N-protonated form is found to be more stable than the NH₂- and CO-protonated forms by 60 and 108 kJ/mol, respectively while for 3HPA, the N-protonated form is more stable than the CO-protonated form by 92 kJ/mol. In the case of 4NiAn we have compared the basicity of the amine function with that of the nitro group and found the NO₂ function more basic than the NH₂

function by 108 kJ/mol. For the IR-MALDI matrices, the most basic sites are the oxygen atom of the carbonyl group for urea and of the hydroxyl group in position 1 for glycerol. For this later, the proton is better stabilized in interaction between both oxygens of the primary hydroxyl groups via a six-membered ring than in interaction between an oxygen of a primary hydroxyl group and the oxygen of the secondary hydroxyl group via a five-membered ring. The geometries of the most stable structures of protonated matrices are given in Fig. 4. The Proton affinities evaluated at the highest level of theory are given in Table 1. There is a very good agreement between the calculated and available experimental data except for G. Our calculation results differ by less than 2.5% from available experimental determinations except for G for which the difference is 3.2%. However, the “experimental” PA of glycerol given in the NIST data base [35] was not experimentally determined but approximated from values available for triamines [37]. The PA of matrices are between 937 (2ANA) and 847 (G) kJ/mol. The order of proton affinity is the following:



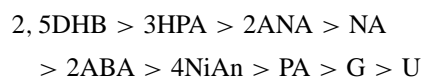
In the literature, the ionization models [24,33,34, 38,39] proposed for explaining the formation of protonated and deprotonated sample molecules involve the acid–basic properties of matrices. Experimental results [26] indicate that PA is a better matrix than 3HPA for the deprotonation of oligonucleotides, in agreement with its greater proton affinity.

3.2. Proton affinities of deprotonated molecules

In the gas phase, by definition, the proton affinity of a deprotonated acid corresponds to the ΔH_{acid} of the neutral molecule. In the studied molecules, the functional groups able to loss a proton are: the carboxyl, the hydroxyl and the amine functions. For 2,5DHB, 2ANA, 3HPA, 2ABA and G, a competition exists between various deprotonation sites. At the B3LYP/6-31+G** level of theory, the carboxyl function is calculated to be more acidic than the amine

function by 102 and 82 kJ/mol for 2ANA and 2ABA, respectively. An interesting result concerns the relative acidity of the carboxyl and hydroxyl groups in the *ortho*-position on the aromatic ring of 2,5DHB and 3HPA. Both functional groups have the same acidity in the case of 2,5DHB while the carboxylate does not exist in the case of 3HPA: starting from a structure of $[\text{3HPA-H}]^-$ in which a hydrogen bond may exist between the carboxylate and the hydrogen of the hydroxyl group in *ortho*-position, geometry optimization leads to the hydroxylate form; without hydrogen bond, the carboxylate form is calculated to be less stable than the hydroxylate form by 135 kJ/mol. On the other hand, the hydroxyl of 2,5DHB in position *meta* is less acid than the others by 64 kJ/mol.

The geometries of the most stable structure of deprotonated matrices are given in Fig. 5. The ΔH_{acid} of matrices are given at the highest level of theory in Table 1. There is an excellent agreement between the calculated and available experimental data. Our calculation results differ by <1.5% (often by <0.5%: 2,5DHB, NA and U) from available experimental data. The matrix found to be the most acidic is 2,5DHB (1367 kJ/mol) while the less acidic is urea (1519 kJ/mol). The calculations give 3HPA and 4NiAn slightly more acidic than do the experiment. The scale of acidity is the following:



This scale gives rise to some comments:

- the most acidic matrices are the aromatic acids substituted in *ortho* by a hydroxyl group;
- the most acidic matrices include the matrices very often used in the positive mode;
- some matrices (NA, 2ANA) exhibit an amphoteric character with good basicity and good acidity and used either in the positive or in the negative mode.

3.3. Ionization energies

The formation of matrix radical cations by multi-photon ionization is considered by many authors as

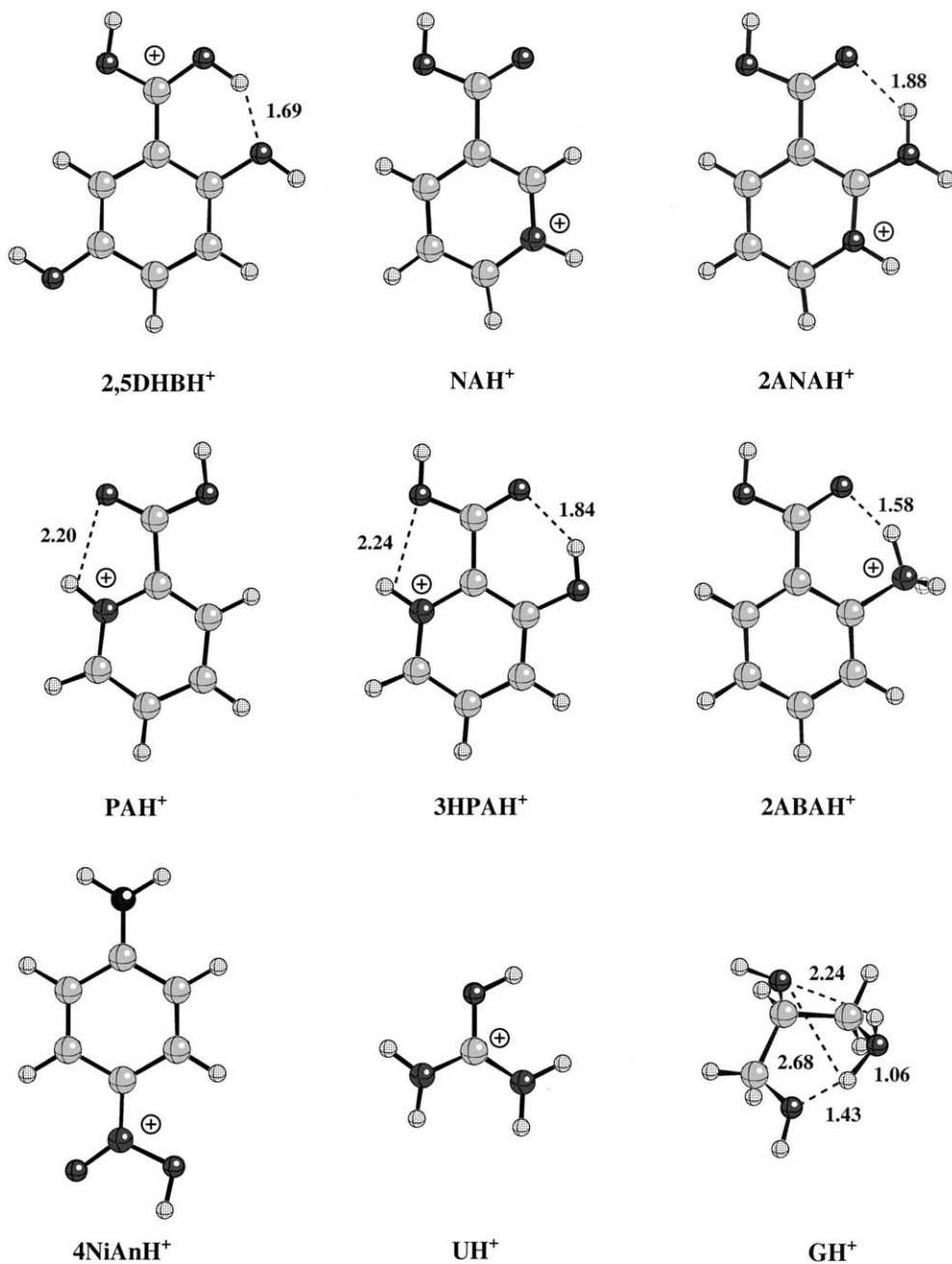


Fig. 4. Optimized geometries (B3LYP/6-31+G** level) of the most stable structures of protonated matrices.

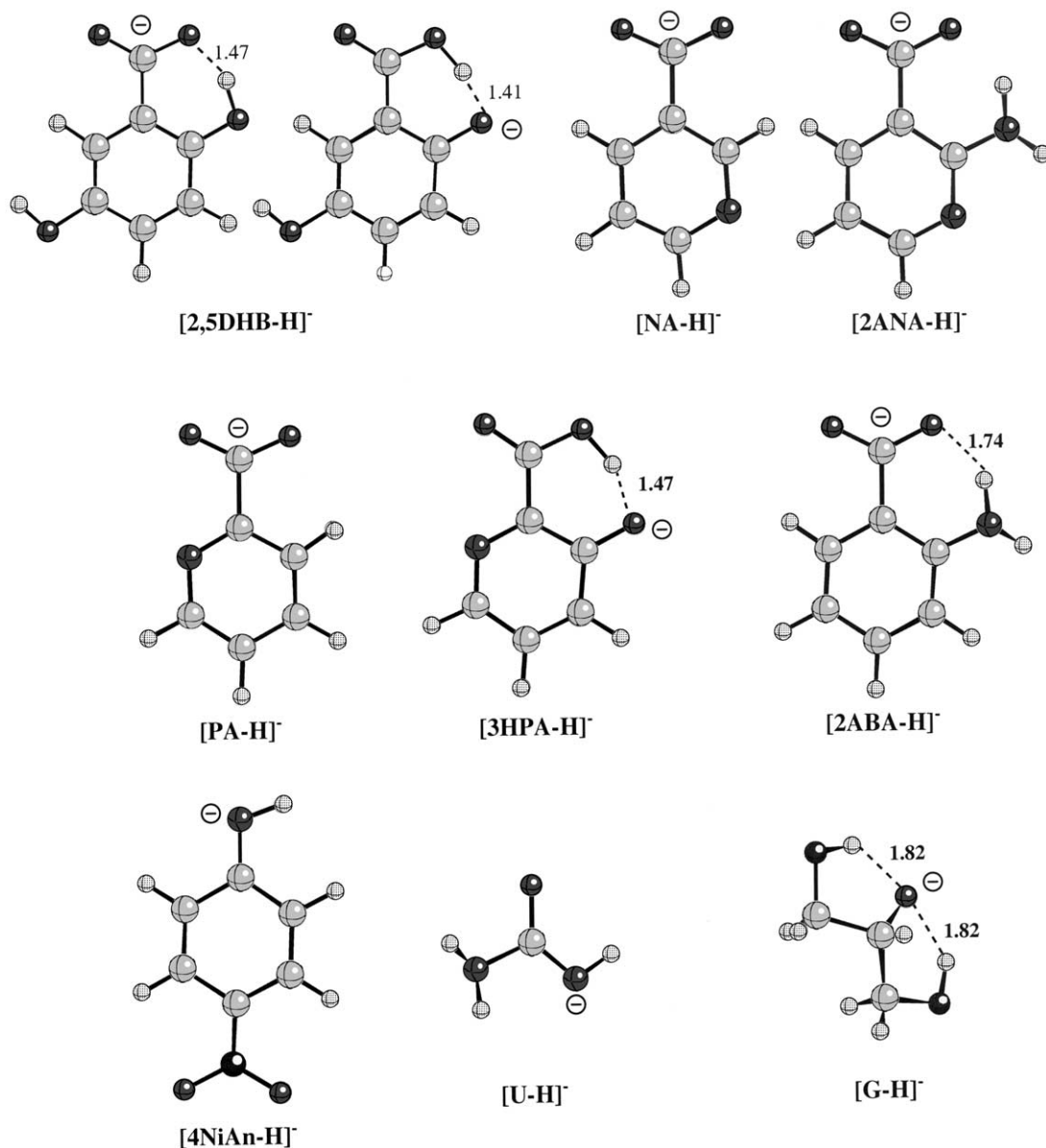


Fig. 5. Optimized geometries (B3LYP/6-31+G** level) of the most stable structures of deprotonated matrices.

a strong candidate for the UV-MALDI primary ionization step. From a theoretical point of view, irradiances used in UV-MALDI are too low to allow significant absorption of more than two photons. If the IE of the matrix is greater than 7.33 eV, the energy of two photons of a N₂ laser (laser most currently used

for MALDI experiments), ionization could nevertheless occur assisted either by a “photothermal” effect [40] or by “energy pooling” [34]. However, the lower the lack of energy, the easier the ionization of the matrix. An efficient matrix should have an IE as low as possible to easily get over the primary ionization step.

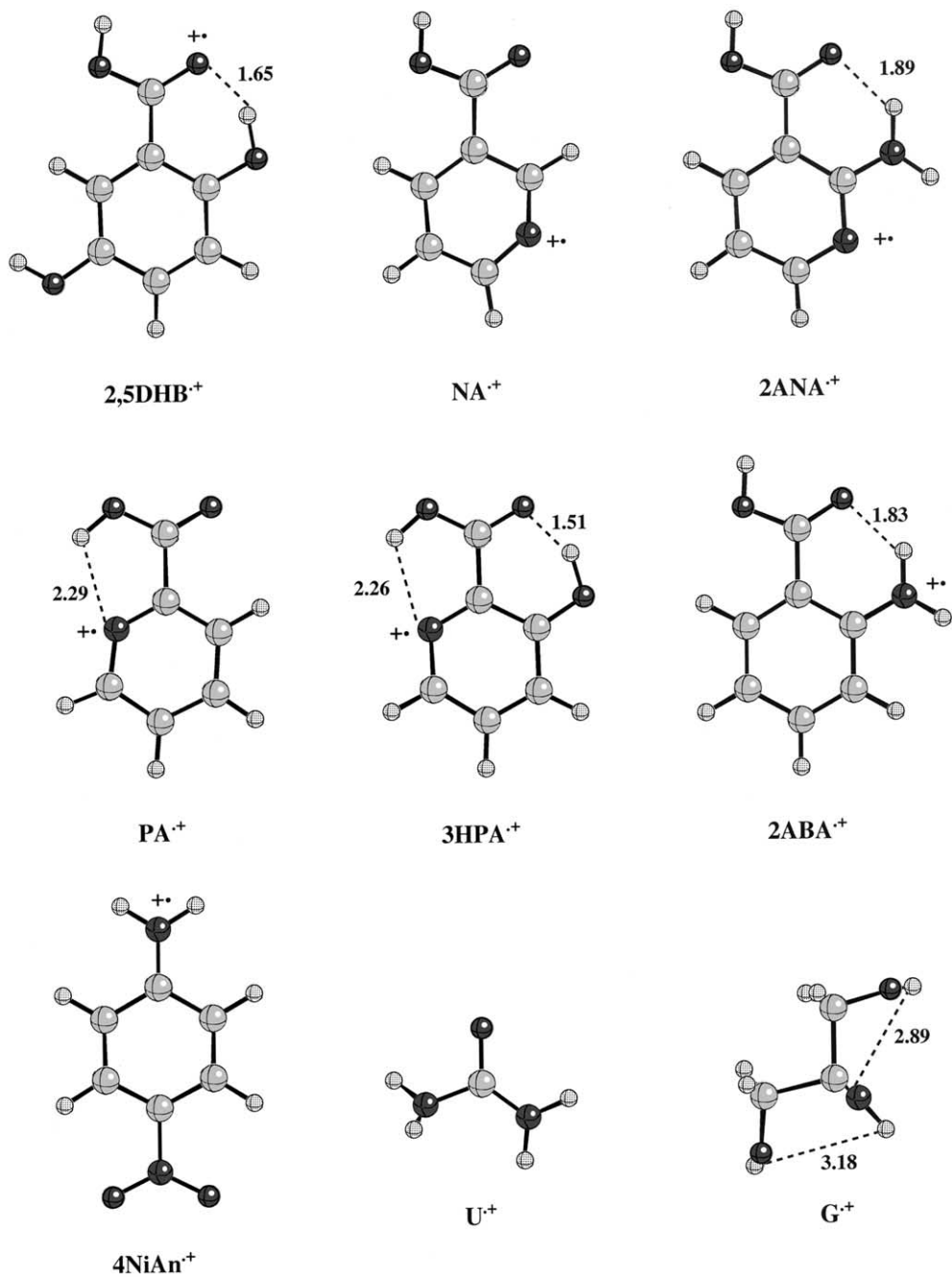


Fig. 6. Optimized geometries (B3LYP/6-31+G** level) of the most stable structures of molecular radical cations.

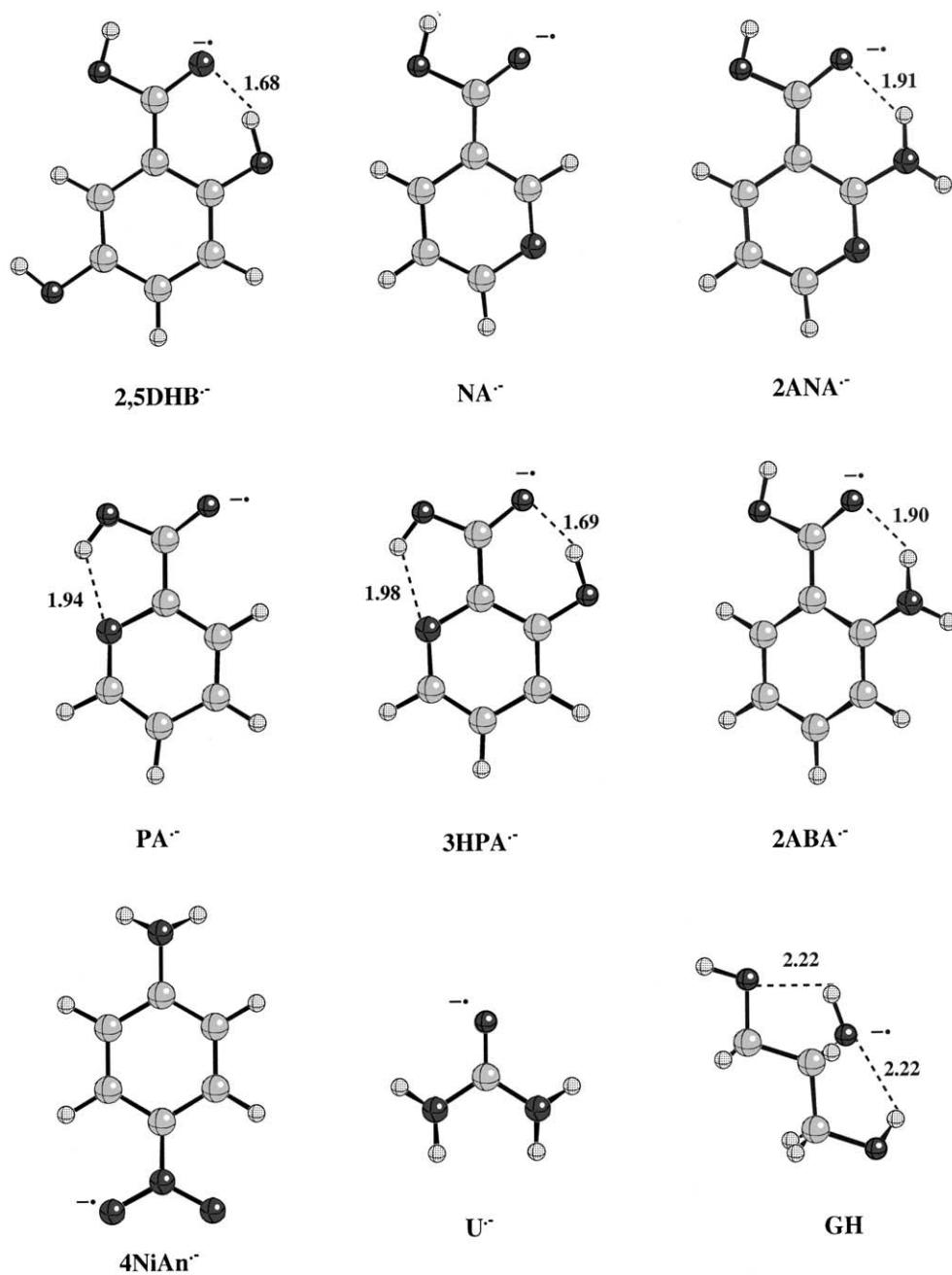
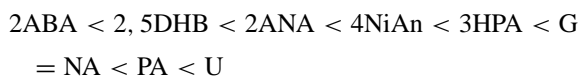


Fig. 7. Optimized geometries (B3LYP/6-31+G** level) of the most stable structures of radical anions.

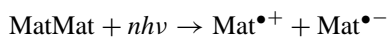
The geometries of the most stable structure of matrix radical cations are given in Fig. 6. The IE of various matrices are given at the highest level of theory in Table 1. The UV-matrix found to have the lowest IE is 2ABA (7.72 eV) while PA is found to have the highest (9.27 eV). The scale of IE is the following:



The calculated values are about 2% lower than the available experimental values.

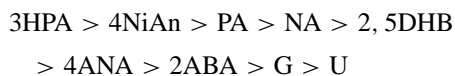
3.4. Electron affinities

Disproportionation reactions have been suggested to explain the simultaneous formation of positive and negative ions in MALDI experiments [33,39]. One of these reactions associated with the primary ionization step of the matrix (Mat) consists in electron transfer between two coupled molecules undergoing laser irradiation following the equation:



The enthalpy associated with such a reaction (in the gas phase) is the difference in energy between the IE and the EA of the matrix. The greater the EA, the smaller the reaction enthalpy.

The geometries of the most stable structure of matrix radical anions are given in Fig. 7. The EA of the various matrices are given at the highest level of theory in Table 1. The UV-matrix found to have the highest EA is 3HPA (1.0 eV) while U is found to have the lowest (−0.85 eV). The scale of EA is



The last column of Table 1 gives the difference in energy between the IE and the EA of each matrix. It may be expected that the smaller this difference, the easier the UV-MALDI primary ionization step. The smallest difference is calculated for 2,5DHB and 4NiAn (7.3 eV). Three other matrices 2ABA and 3HPA have enthalpies of reaction below 8 eV.

4. Conclusions

This work shows that molecular orbital calculations performed with a B3LYP functional density approach can be accurate to evaluate thermochemical data on molecules with several heavy atoms: our calculation results differ by less than 2.5% (except G, see text) from available experimental determinations. Numerous unknown thermochemical values were determined: PA of neutral 2ANA, 2ABA and U; PA of deprotonated 2ANA, 2ABA, PA and G; IE of 2ANA, 2ABA, PA, 3HPA and G; EA of the nine studied matrices. The most basic matrices are aniline derivatives. The most acidic matrices are those with the aromatic ring substituted in *ortho* by a carboxylic and a hydroxyl group. The acidity of the hydroxyl in *ortho* is interesting. It is equal to that of the carboxylic group in 2,5DHB and greater in 3HPA. Some matrices (NA, 2ANA) exhibit an amphoteric character with good basicity and good acidity. The difference in energy between the IE and EA of matrices could be useful to better understand the UV-MALDI primary ionization step.

References

- [1] (a) M. Karas, D. Bachmann, F. Hillenkamp, *Anal. Chem.* 57 (1985) 2935; (b) M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, *Int. J. Mass Spectrom. Ion Processes* 78 (1987) 53.
- [2] R. Zenobi, R. Knochenmuss, *Mass Spectrom. Rev.* 17 (1998) 337.
- [3] R. Knochenmuss, A. Stortelder, K. Breuker, R. Zenobi, *J. Mass Spectrom.* 35 (2000) 1237.
- [4] M. Karas, M. Glückmann, J. Schäfer, *J. Mass Spectrom.* 35 (2000) 1.
- [5] S. Bourcier, S. Bouchonnet, Y. Hoppilliard, *Int. J. Mass Spectrom.* 210/211 (2001) 59.
- [6] L.A. Curtiss, K. Raghavachari, G.W. Trucks, J. Pople, *J. Chem. Phys.* 94 (1991) 7221.
- [7] L.A. Curtiss, K. Raghavachari, J. Pople, *J. Chem. Phys.* 98 (1993) 1293.
- [8] B.J. Smith, L. Radom, *J. Phys. Chem.* 99 (1995) 6468.
- [9] B.J. Smith, L. Radom, *Chem. Phys. Lett.* 245 (1995) 123.
- [10] G.N. Merill, S.R. Kass, *J. Phys. Chem.* 100 (1996) 17465.
- [11] D. Yu, A. Rauk, D.A. Armstrong, *J. Am. Chem. Soc.* 117 (1995) 1789.
- [12] I.A. Topol, S.K. Burt, N. Russo, M. Toscano, *J. Am. Soc. Mass Spectrom.* 10 (1999) 318.

- [13] A.D. Becke, *J. Chem. Phys.* 98 (1993) 5648.
- [14] J. Catalan, J. Palomar, J.L.G. de Paz, *Int. J. Mass Spectrom. Ion Processes* 175 (1998) 51.
- [15] P. Burk, I.A. Koppel, I. Koppel, I. Leito, O. Travnikova, *Chem. Phys. Lett.* 323 (2000) 482.
- [16] L.A. Curtiss, P.C. Redfern, K. Raghavachari, J. Pople, *J. Chem. Phys.* 109 (1998) 42.
- [17] Gaussian 94 (Revision B.1), M.J. Frisch, G.W. Trucks, H.B. Schlegel, P.M.W. Gill, B.G. Johnson, M.A. Robb, J.R. Cheeseman, T.A. Keith, G.A. Petersson, J.A. Montgomery, K. Raghavachari, M.A. Al-Laham, V.G. Zakrzewski, J.V. Ortiz, J.B. Foresman, J. Cioslowski, B.B. Stefanov, A. Nanayakkara, M. Challacombe, C.Y. Peng, P.Y. Ayala, W. Chen, M.W. Wong, J.L. Andres, E.S. Replogle, R. Gomperts, R.L. Martin, D.J. Fox, J.S. Binkley, D.J. Defrees, J. Baker, J.J.P. Stewart, M. Head-Gordon, C. Gonzalez, J.A. Pople, Gaussian, Inc., Pittsburgh, PA, 1995.
- [18] Gaussian 98 (Revision A.7), M.J. Frisch, et al. (see [17]), Gaussian, Inc., Pittsburgh, PA, 1998.
- [19] M. Karas, U. Bahr, A. Ingendoh, E. Nordhoff, B. Stahl, K. Strupat, F. Hillenkamp, *Anal. Chim. Acta* 241 (1990) 175.
- [20] K. Strupat, M. Karas, F. Hillenkamp, *Int. J. Mass Spectrom. Ion Processes* 111 (1991) 89.
- [21] M. Karas, F. Hillenkamp, *Anal. Chem.* 60 (1988) 2299.
- [22] F. Hillenkamp, M. Karas, in: P. Longevialle (Ed.), *Advances in Mass Spectrometry*, Heyden & Son, London, 1989, pp. 416–417.
- [23] A. Overberg, M. Karas, U. Bahr, R. Kaufmann, F. Hillenkamp, *Rapid Commun. Mass Spectrom.* 4 (1990) 293.
- [24] H. Ehring, M. Karas, F. Hillenkamp, *Org. Mass Spectrom.* 27 (1992) 472.
- [25] K.J. Wu, A. Steding, C.H. Becker, *Rapid Commun. Mass Spectrom.* 7 (1993) 142.
- [26] K. Tang, N.I. Taranenko, S.L. Allman, C.H. Chen, L.Y. Chang, K.B. Jacobson, *Rapid Commun. Mass Spectrom.* 8 (1994) 673.
- [27] M.C. Fitzgerald, G.R. Parr, L.M. Smith, *Anal. Chem.* 65 (1993) 3204.
- [28] C.M. Nelson, K. Crellin, J. Berry, J.L. Beauchamps, L.M. Smith, in: A.B. Giordani (Ed.), *Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics*, Portland, 1996, p. 733.
- [29] R.D. Burton, C.H. Watson, J.R. Eyler, G.L. Lang, D.H. Powell, M.Y. Avery, *Rapid Commun. Mass Spectrom.* 11 (1997) 443.
- [30] R.J.J.M. Steenvoorden, K. Breuker, R. Zenobi, *Eur. Mass Spectrom.* 3 (1997) 339.
- [31] T.J.D. Jorgensen, G. Bojesen, H. Rahbek-Nielsen, *Eur. Mass Spectrom.* 4 (1998) 39.
- [32] M. Mormann, S. Bashir, P. Derrick, D. Kuck, *J. Am. Soc. Mass Spectrom.* 11 (2000) 544.
- [33] K. Breuker, R. Knochenmuss, R. Zenobi, *Int. J. Mass Spectrom.* 184 (1999) 25.
- [34] V. Karbach, R. Knochenmuss, *Rapid Commun. Mass Spectrom.* 12 (1998) 968.
- [35] NIST Chemistry Web Book, NIST Standard Reference Database Number 69, February 2000 Release, available at webbook.nist.gov/chemistry
- [36] E.P.L. Hunter, S.G. Lias, *J. Phys. Chem. Ref. Data* 27 (1998) 413–428.
- [37] M. Meot-Ner, P. Hamlet, E.P. Hunter, F.H. Field, *J. Am. Chem. Soc.* 102 (1980) 6393.
- [38] P.C. Liao, J. Allison, *J. Mass Spectrom.* 30 (1995) 408.
- [39] R. Knochenmuss, V. Karbach, U. Wiesli, K. Breuker, R. Zenobi, *Rapid Commun. Mass Spectrom.* 12 (1998) 529.
- [40] D.A. Allwood, P.E. Dyer, R.W. Dreyfus, *Rapid Commun. Mass Spectrom.* 11 (1997) 499.