

Protonation of caffeine: A theoretical and experimental study



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ABSTRACT

Protonation of caffeine was examined by ion mobility spectrometry equipped with two ionization sources, corona discharge (CD) and UV photoionization. Three peaks were observed in ion mobility spectrum by simultaneously running the two ionization sources. Experimental and theoretical evidence was collected to link the observed peaks to caffeine related ionic species. One peak was attributed to the M^+ ion while the other two were assigned to different protonated isomers of caffeine. In the case of CD ionization source, it was observed that different sites of caffeine compete for protonation and their relative intensities, depends on the sample concentration as well as the nature of the reactant ions. The new concept of “internal proton affinity” (IPA) was defined to express the tendency of holding the added proton for each atom in a molecule.

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1. Introduction

The detection and measurement of caffeine (CAF) or trimethylxanthine ($M = 194$), as a central nervous system and metabolic stimulant [1], have attracted the attention of many researchers. Chromatography and mass spectrometry with different ionization sources have been widely used in the study of caffeine. Electrospray ionization mass spectrometric analysis of caffeine showed only one major peak at m/z 195, corresponding to non-fragmented protonated caffeine, MH^+ [2–4]. Atmospheric pressure photoionization (APPI), using a dopant, also exhibited only one major peak for caffeine at m/z 195 [5]. In addition, mass spectrum of caffeine with a nickel-63 ionization source showed two peaks for caffeine, corresponding to M^+ and MH^+ [6], while with corona discharge ionization source, only one peak at m/z 195 was shown [7]. Furthermore, determination of caffeine by means of LC–MS/MS with electrospray ionization in human plasma [8] and breast milk [9] showed only the protonated form of caffeine as non-fragmented product ion.

In addition to the above techniques, ion mobility spectrometry (IMS) has been used to measure caffeine in dietary supplement [10] and chewing gum [11]. Ion mobility spectrum of caffeine with electrospray ionization source showed only one product ion peak [12,13]. Distributed plasma ionization source (DPIS) for ion mobility spectrometry also led to one product ion peak for caffeine [14]. An old IMS–MS study of caffeine with radioactive ionization source at room temperature reviled four peaks, mainly nitrogen clusters of MH^+ and M_2^+ and an unknown ion at $m/z = 252$ [15].

Proton affinity (PA) is one of the most important thermodynamic quantities that link the thermochemistry of ions to that of the neutral molecules. The importance of proton transfer reactions in chemistry and biochemistry made the researchers to be increasingly involved in theoretical calculation and experimental measurement of proton affinities and gas phase basicities (GB). There are several experimental techniques, based on either chemical equilibrium or kinetic measurements, for determining the relative proton affinities. The techniques, including high-pressure mass spectrometry [16,17], fast atom bombardment mass spectrometry [18,19], Fourier-transform mass spectrometry [20], selected ion flow tube [21,22] and ion mobility spectrometry [23], can be used for proton affinity determination.

In the case of multifunctional molecules, such as caffeine (Fig. 1), there may be several sites for protonation. Experimental methods explore only the most stable protonated structures corresponding to the most basic sites in the molecule such that the topical proton affinities of less basic sites are missing in the experimental studies. Therefore, most experimental measurements of proton affinities should basically be supported by theoretical calculations [24–28]. The effect of molecular structure on proton affinity has been investigated in several papers [27,29,30]. For example, Wolken and Turecek [31] reported 25 theoretical proton affinities for uracil tautomers. The proton affinity of uracil obtained from the *ab initio* calculations for the most basic site was in good agreement with that derived from experimental measurements.

Here, we report a joint theoretical and experimental investigation of proton affinities of CAF as a test compound. In this study, we used an ion mobility spectrometer (IMS) for experimental measurements. The advantage of IMS is that unlike mass spectrometer, different protonated molecules with similar masses may be

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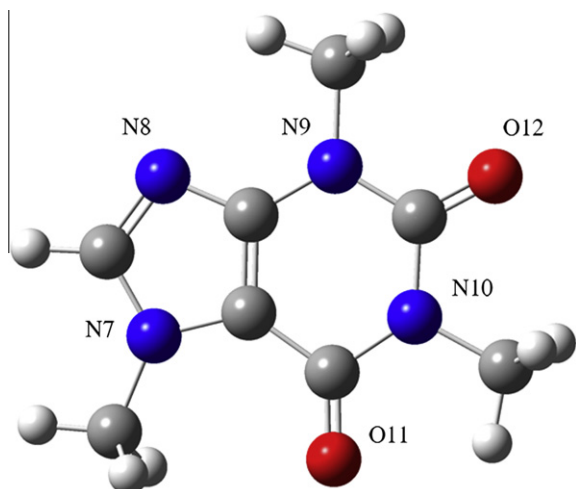


Fig. 1. Molecular structure of caffeine.

separated in IMS [32,33] because the size and the shape of the ion is also important in IMS.

All mass spectrometric studies at elevated temperatures, using corona discharge or electrospray ionization sources, revealed that the m/z 195, corresponding to MH^+ ion, appears in mass spectra. However, we observed two distinct peaks in ion mobility spectrum of CAF. The main objective of this work is to assign these two peaks to different isomers of MH^+ with the aid of calculated gas-phase thermochemical properties of CAF ion and its protonated cations. Additional experimental observations, obtained by using UV source and ion molecule reactions, are used to support the assignment and confirm the theoretical calculations.

2. Experimental

2.1. Instrument

All experiments were performed with an ion mobility spectrometer, constructed in our laboratory at Isfahan University of Technology [34]. To summarize, a continuous current of ions is pulsed by a shutter grid. The ion packet drifts under a constant electric field at atmospheric pressure. Ions are then separated in the drift region, based on their mobility, which depends on their mass, charge and size. The IMS instrument used in this study was equipped with two ionization sources: corona discharge (CD) and UV photoionization. The details of the UV ionization source and the geometry of the two sources as well as the experimental method have been given in Refs. [35,36]. For photoionization, a UV lamp was mounted parallel to the axis of the ion mobility cell. The corona discharge electrode was mounted perpendicular to the UV radiation. The design allows one to observe peaks from either the corona discharge or photoionization both individually and simultaneously. Thus, it is possible to compare accurately the peaks in the ion mobility spectra of each individual source.

The optimized experimental conditions are given in Table 1.

2.2. Sample preparation

Caffeine was purchased from Merck and used without further purification. Standard solutions of caffeine in water were prepared and working solutions in the range of 5–200 ppm were prepared by consecutive dilution. 2 μ L of the working solution was loaded into the injection port and the solvent was allowed to evaporate. Analytical grade toluene in vapor form was used as the dopant in

Table 1
The optimized experimental conditions.

Parameter	Setting
Length of drift tube	11 (cm)
Drift field	466 (V/cm)
Flow rate of drift gas (N_2)	800 (mL/min)
Flow rate of carrier gas (N_2)	300 (mL/min)
Flow rate of dopant (toluene)	300 (μ L/min)
Injection port temperature	220 ($^{\circ}$ C)
IMS cell temperature	200 ($^{\circ}$ C)
Polarity	Positive

the case of UV photoionization. Ammonia, in gas phase, was prepared through head space released from solid ammonium carbonate. A syringe pump (New Era Pump System Inc. USA) was used to inject headspace vapor of toluene or ammonia into the ionization region at a flow rate of 300 and 400 μ L/min, respectively.

2.3. Computational details

The theoretical calculations have been carried out with the quantum computational GAUSSIAN 09 package [37]. All of the calculations have been performed at two different levels of theory including Hartree–Fock (HF) and density functional theory (DFT). The geometry of the neutral, cation and different protonated CAF isomers was optimized at B3LYP, CAM-B3LYP and wB97X levels of theory using 6-311++G** basis set separately. The frequency calculations were performed on the optimized structures to search for the negative frequency and obtain the thermodynamic values such as enthalpy, Gibbs free and internal energy of the considered species. In addition, the volumes of the ions were calculated using the structures optimized at HF/aug-cc-pvdz and B3LYP/6-311++G** levels of theory.

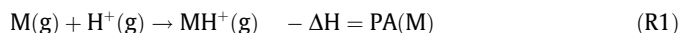
3. Results and discussion

3.1. Thermodynamic stability of isomers and proton affinity

Fig. 1 shows the molecular structure of CAF. As can be seen, there are several sites including the oxygen of carbonyl groups and the nitrogen of imine group (N8) for protonation.

To find the most stable protonated isomer of CAF, the neutral molecule was protonated from different sites (all nitrogen and oxygen). The geometry of the protonated isomers was optimized to select the most stable structures. The results of the geometry optimization for the most three stable isomers, relative electronic and Gibbs free energy, are reported in Table 2. The structure, protonated from the N8 site, $MH^+(N8)$, is the most stable one among the ions. The next stable isomers, $MH^+(O11)$ and $MH^+(O12)$, are about 5 kcal/mol less stable than the N8 protonated CAF. It should be noticed that the difference in the stability of the two oxygen protonated isomers is small, which is related to similar chemical environment around the oxygen atoms in the two isomers. The optimized structures of the protonated isomers reported in Table 2 were used for further theoretical studies.

The proton affinity (PA) of a molecule M is the negative of the enthalpy change of the protonation reaction:



The calculated enthalpies of the components contributing in reaction R1 were used to obtain the PA of CAF. Frequency calculations were performed on each component at 473 K, the temperature at which the ion mobility spectra were recorded. The enthalpy of proton at 473 K (2.331 kcal/mol) was taken from Ref. [38]. Table 3 reports the PA of the CAF when it is protonated from

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