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# Infrared multiple photon dissociation spectroscopy of ciprofloxacin: Investigation of the protonation site

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ABSTRACT

The vibrational spectrum of isolated protonated ciprofloxacin was recorded in the range 1100–2000 $cm^{-1}$ by means of infrared multiple photon dissociation (IRMPD) spectroscopy. The spectrum was obtained by electrospraying a methanol solution of ciprofloxacin in a Paul ion trap, coupled to the tunable IR radiation of a free electron laser. This spectroscopic study has been complemented by quantum chemical calculations at the DFT and MP2 levels of theory to identify the possible structures present under our experimental conditions. Several low-energy isomers with protonation occurring at the piperazinyl amino group and at the carbonyl group are predicted in the energy range 0–84 kJ $mol^{-1}$. A good agreement between the measured IRMPD spectrum and the calculated absorption spectrum is observed for the isomer protonated at the piperazinyl amino group. This isomer is calculated at MP2 level of theory to lie about 76 kJ/mol above the most stable isomer which is protonated at the quinone carbonyl group. This discrepancy can be rationalized by assuming that the protonation at the piperazinyl amino group, typical of the zwitterionic form that is found in protic solvents, is retained in the ESI process.

The vibrational bands observed in the IRMPD spectrum are assigned to normal modes of the isomer protonated at the piperazinyl amino group, with deviations of less than 20 $cm^{-1}$ between measured and calculated frequencies.



## 1. Introduction

Quinolones, a commonly used term for the quinolone carboxylic acids or 4-quinolones, are a class of synthetic broad spectrum antibiotics [1,2]. The majority of quinolones used for bacterial infections belong to the class of fluoroquinolones, which have a fluorine atom attached to the central ring system. Ciprofloxacin (CIP) (Fig. 1) is one of the most popular drug of this family and is marketed worldwide with over three hundred different brand names. It was the drug of choice for the treatment of victims infected by anthrax [3], after the terrorist attacks of 2001 and subsequent suspected anthrax biological warfare. Like other fluoroquinolone anti-infectives, ciprofloxacin inhibits DNA synthesis in susceptible organisms via inhibition of the enzymatic activities of DNA gyrase and topoisomerase IV [4,5]. The important role of metal ions in the mechanism of action of these drugs is documented in a large number of studies [6,7].

There were numerous attempts to establish the character of the structure of ciprofloxacin [8,9] and its complexes with metal cations [10,11] using various methods including electrochemistry, X-ray diffraction and UV, IR and $^1H$ NMR spectroscopies. These studies have shown that CIP is an amphoteric drug, with four possible species present in aqueous solution: cationic, non-ionic, zwitterionic and anionic, depending on the pH. The zwitterionic species predominates near physiological pH [12]. In organic solvents, ciprofloxacin is non-ionic and in the solid state the molecule exists in zwitterionic form. The protonation site of CIP in condensed phase has been the subject of several investigations [13,14]. CIP contains several proton-binding sites, namely, the amino groups, the pyridone oxygen, and the carboxylate group. The preferred protonation position in the gas-phase has not been determined nor is it immediately obvious. When interacting with metals, CIP can act as a bidentate ligand through the pyridone oxygen and the carboxylate group. Since the behavior in vivo greatly depends on the degree of ionization, lipophilicity, and conformational characteristics, it is important to evaluate the structural factors that could contribute to the drug properties and the extent of its

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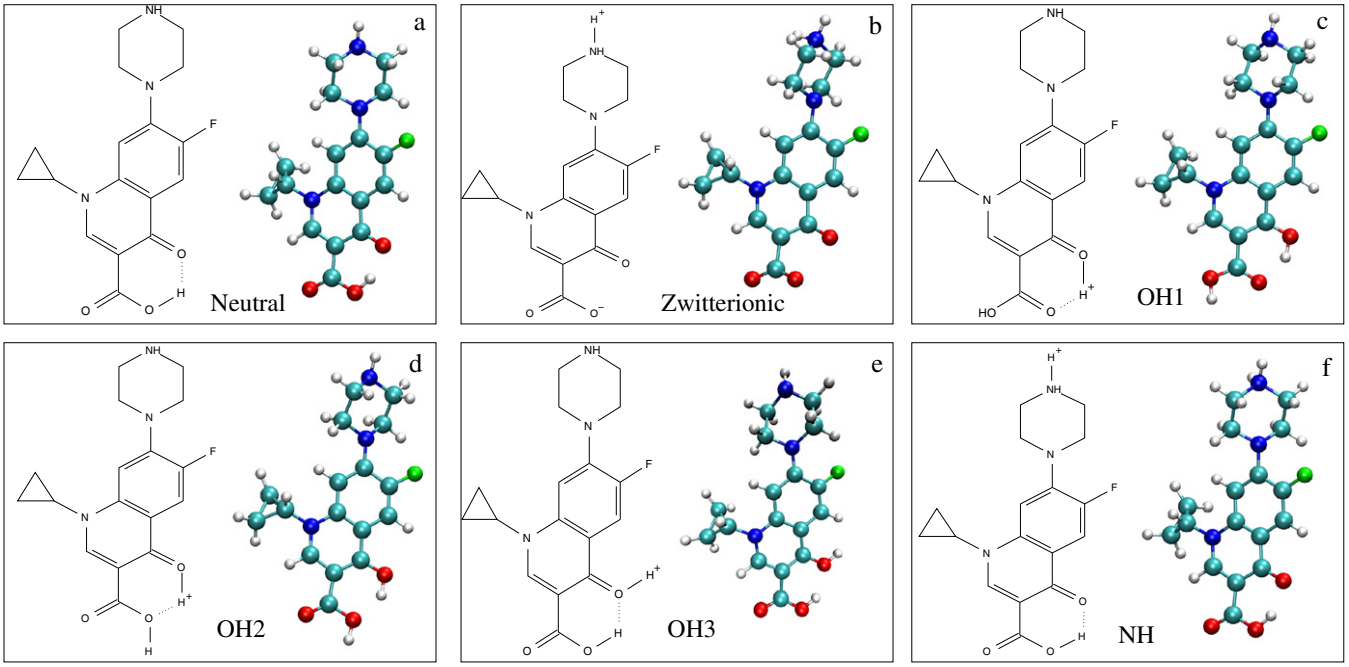


**Fig. 1.** Structures of the most stable isomers of neutral (a) and zwitterionic (b) ciprofloxacin, and OH1 (c), OH2 (d), OH3 (e) and NH (f) isomers of protonated ciprofloxacin, calculated at the B3PW91 level of theory.

interaction with metals. To our knowledge, no direct comparison to gas-phase spectroscopic data has yet been performed to ascertain on the protonation site of isolated CIP. Our aim is to investigate the gas phase structure of protonated ciprofloxacin as part of an ongoing project which aims at studying the mode of binding to metals. Operating in a quite different, highly essential environment, namely the gas phase, presents interesting advantages due to the absence of any interfering effect of the environment, and allowing the comparison with high level theoretical calculations.

It is well established that IRMPD in trapping apparatuses is an excellent tool for the characterization of a variety of gaseous ion species including small organic ions, biomolecular ions (amino acids, peptides, proteins, oligosaccharides), and finally bare and solvated ionic complexes, proton or metal cationized [15–24]. Particular focus on the determination of the preferred site of protonation has been paid [25–28]. The use of IRMPD spectroscopy for obtaining structural information on gaseous ions is gaining widespread recognition and is increasingly exploited, aided by the availability of widely tunable and powerful sources of IR radiation, such as free electron lasers (FEL) and optical parametric oscillator/amplifier (OPO/OPA) laser sources [23,29].

The present study provides an experimental determination of the protonation site of ciprofloxacin in the gas phase through a detailed analysis of the IRMPD spectrum of $CIPH^+$ in the 1100–2000 $cm^{-1}$ range, utilizing quantum chemical calculations at the DFT and MP2 levels of theory.

## 2. Theoretical and experimental methods

### 2.1. Computational details

The minimum energy structures of the various conformers have been calculated by means of geometric optimization using the MP2 and DFT method with various functionals. The starting structure of ciprofloxacin has been taken from the solid state structure reported in Ref. [30].

We have used the following functionals: B3LYP [31], B3PW91 and BPW91 [32] with the 6 + 311G(d, p) basis [33]. The rather tedious exploration of all the various geometries have been performed at the B3LYP level, and we have repeated part of the calculations with the BPW91 and B3PW91 functionals to confirm the B3LYP findings. Calculations with the more extended 6-311 + G(d, p) basis set have been performed with the BPW91 and B3PW91 functionals. An analogue set of optimizations has been carried out using the more computationally demanding MP2 method, albeit only on a small subset of structures, in order to check the possible effect of the dispersion energy. We have used the gaussian09 [34] suite of programs.

For each of the minimum energy structure we have calculated the harmonic vibrational frequencies and the resulting simulated IR absorption spectra. The frequencies obtained at the B3PW91/6-311 + G(d, p) level have been scaled with the formula: $\nu_{(expt)} = 0.955\ \nu_{(calc)} + 25.7$ (in wavenumbers) [25]. The MP2/6-311G(d, p) results have been scaled using a uniform scaling factor of 0.95. Theoretical IR stick spectra have been convoluted with a Gaussian profile with width (FWHM) of 15 $cm^{-1}$, in order to facilitate convenient comparison with the experimental spectrum.

### 2.2. Experimental

IRMPD experiments have been performed at the Centre Laser Infrarouge Orsay (CLIO), using a modified quadupole Paul ion trap (Bruker, Esquire 3000+) coupled to the Free Electron Laser. The IR-FEL beam has been mildly focused in the trap through a ZnSe Brewster window and a conical hole (0.7 mm diameter) in the ring electrode. Multistage mass spectrometry has been performed using the Bruker Esquire Control software. Protonated CIP ions have been mass-selected and accumulated for 1 ms prior to IR irradiation. IRMPD on mass selected and accumulated ions has been performed using the MS2 step, keeping the excitation amplitude zero to avoid any collision-induced fragmentation process. Post-irradiation mass spectra have been recorded after 10 accumulations and this sequence was repeated two times at each photon energy.