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Photophysical properties of gatifloxacin in aqueous solution by laser flash photolysis and pulse radiolysis

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ABSTRACT

GFX in water, at pH 7.0, shows intense absorption bands with peaks at 284 and 333 nm, (ε=24,670 and 12,670 M^{-1} cm^{-1}). Both the absorption and emission properties of GFX were pH-dependent; the pK_a values for the protonation equilibria of the ground state (5.7 and 8.9) and excited singlet state (3.6 and 7.5) of GFX were determined spectroscopically. GFX fluoresces weakly, with a maximum quantum yield for fluorescence emission (0.06) at pH 4.7. A series of experiments were performed to characterize the transient species of GFX in aqueous solution using laser flash photolysis and pulse radiolysis. GFX undergoes monophotonic photoionization with a quantum yield of 0.16 on a 355 nm laser excitation. This process leads to the formation of a long-lived cation radical with a maximum absorption at 380 nm. Triplet–triplet absorption had maximum absorption at 510 nm. The reaction of GFX with one-electron oxidant $N_3^{\bullet}$ was investigated and the bimolecular rate constant was determined to be 3.1×10^9 M^{-1} s^{-1}.

1. Introduction

Fluoroquinolone compounds (FQs) are successful antimicrobials widely prescribed as antibiotics (Rubinstein, 2001; Andersson and MacGowan, 2003). Their pharmacological action consists in specific inhibition of subunit-A of the bacterial topoisomerase DNA gyrase, which controls the shape of DNA (Domagala et al., 1986). However, a few serious side effects have been reported (Wolfson and Hooper, 1989; Chu and Fernandes, 1991; Mitsushashi, 1992; Condorelli et al., 1996; Martinez and Chignell, 1998; Tokura et al., 1996; Klecak et al., 1997) with FQs.

Many of the studies have indicated that the mechanism of phototoxicity was through the production of ionic radicals and/or the singlet oxygen and other reactive oxygen species (ROS) formed in the photochemical processes (Umezawa et al., 1997; Martinez et al., 1998; Wagai and Tawara, 1992). Apart from phototoxicity, there are many studies on the photochemical properties of FQs especially on the photo-defluorination process due to the strength of the aromatic C–F bond (dissociation energy *ca.* 120 kcal mol^{-1}) (Fasani et al., 1998; Cuquerella et al., 2006a,2006b; Fasani et al., 2010,2009; Cuquerella et al., 2004; Albini and Monti, 2003). Photoreactivity may be initiated from the excited singlet state (Hayashi, 2005; Zuo et al., 1992). Therefore, other species (dissolved in the photoreaction), which can quench excited state, may affect the photoreactivity of FQs. This is the case in phosphate, commonly used as a buffer, which has been shown to quench the excited singlet and triplet states of lomefloxacin, norfloxacin and enoxacin (Zuo et al., 1992; Cuquerella et al., 2006a,2006b) through static interaction with the positively charged 4'-NH_2^+ moiety in the piperanizyl ring present in these FQs.

The structure of gatifloxacin [(±)-1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, GFX] is shown in Chart 1. It is the fourth generation of a new class of synthetic antibacterial FQ agents. It shows activity against Gram-positive, Gram-negative and anaerobic species. It also shows good oral absorption, fewer adverse effects and low phototoxicity (Zheng and Liang, 2005). It acts intravenously by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV (Perry et al., 1999). This FQ is mainly excreted unaltered in urine (>75%), and no pharmacokinetic differences have been observed between oral and intravenous administration (Zhanel and Noreddin, 2001).

Several analytical techniques have been utilized to determine the phototoxicity of GFX, such as high-performance liquid chromatography (Overholser et al., 2003; Nguyen et al., 2004; Maria et al., 2006; Salgado and Lopes, 2006; Vishwanathan et al., 2001),

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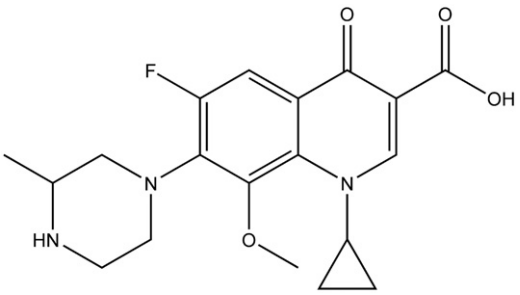

Chart 1. Structure of GFX.

spectrophotometry (Venugopal and Saha, 2005; Amin et al., 2006; Salgado and Oliveira, 2005), high-performance thin-layer chromatography (Motwani et al., 2006; Suhagia et al., 2006), chemiluminescence (Lian et al., 2002), high-performance capillary electrophoresis (Zhu et al., 2002) and microbiological assay (Salgado et al., 2006). However, a systematic investigation on the photochemical properties of GFX was not reported yet. In this paper, photophysical properties of GFX were investigated using steady-state and time-resolved techniques such as laser flash photolysis and pulse radiolysis. The fluorescence spectra and ultraviolet spectra showed that the absorption and emission properties of GFX were pH-dependent. GFX fluoresces weakly, the quantum yield for fluorescence emission being maximum (0.06) at pH 4.7. The 355 nm laser flash photolysis showed a long-lived cation radical at 380 nm, with a triplet state absorption at 510 nm. Moreover, GFX could react with $N_3^{\bullet}$, and produce its cation radical. Then the cation radical can oxidize tryptophan by pulse radiolysis.

2. Materials and methods

2.1. Material

High purity (> 99.8%) GFX was obtained from Sigma Chemicals and used as received. Phosphate salts and amino acids were obtained from J&K Chemical Ltd. All solutions were prepared freshly with ultrapure water provided by a Millipore purification system.

2.2. Methods

2.2.1. Absorption and emission measurements

UV–visible (UV–vis) absorbance was measured on model U-3900/3900H spectrophotometer (Hitachi High-Technologies Corporation). Fluorescence signals were measured on model F-4500 luminescence spectrometer (Hitachi High-Technologies Corporation). Fluorescence quantum yield were determined by comparison with quinine bisulfate in 0.05 M sulfuric acid (Φ_F=0.56 (Lian et al., 2002)) as a standard.

A 2×10^{-5} M solution of GFX was prepared in water and 0.1 M KCl was added in order to keep the ionic strength constant during the titration. The pH of the solution was modified by adding drops of concentrated HCl or NaOH solution, under continuous stirring. PH was constantly monitored using a glass electrode and the absorption and fluorescence were measured immediately after each addition. The titration curves were fitted using a sigmoidal function and analyzed by nonlinear least square methods. The pK_a values were determined from the half-height of the titration curve.

2.2.2. Laser flash photolysis and pulse radiolysis

Laser flash photolysis (LFP) experiments were carried out at the Shanghai Institute of Applied Physics using Nd: YAG laser. Nd: YAG laser provides a 355 nm pulse with duration of 5 ns and the maximum energy of 240 mJ per pulse was used as the pump light source. A Xenon lamp was employed as detecting light source. The laser and the analyzing light beam passed perpendicularly through a quartz cell. The transmitted light entered a monochromator equipped with an R955 photomultiplier. The output signal from the HP54510B digital oscillograph was transferred to a personal computer for further analysis. The LFP setup has been previously described (Zuo et al., 1992). All experiments were performed in aqueous solution. Samples were bubbled with high-purity (99.999%) N_2, or O_2 (99.999%) for at least 20 min, before photolysis experiments were initiated.

Pulse radiolysis experiments were performed utilizing a 10 MeV linear accelerator, which delivers an electron pulse with duration of 10 ns. The dosimetry of electron pulse was determined by thiocyanate dosimeter using G $[(SCN)_2^{\cdot-}]$=5.8 in a 0.1 mM KSCN saturated with N_2O by taking $\varepsilon_{480\ nm}$=7,600 $M^{-1}\ cm^{-1}$ for $(SCN)_2^{\cdot-}$. The details of the setup and operation conditions were given in the previous paper (Yao et al., 1995). The dose per electron pulse was 10 Gy.

3. Results and discussion

3.1. Absorption and emission properties

The UV–vis absorption spectra of GFX at pH 7.4 showed two bands centered around 284 and 333 nm, with molar absorption coefficients of 24,670 and 12,670 $M^{-1}\ cm^{-1}$, respectively. Similar values have been reported for other FQs such as ofloxacin (Navaratnam and Claridge, 2000), enoxacin (Sortino et al., 1998), moxifloxacin (Lorenzo et al., 2008) and sarafloxacin (Lorenzo et al., 2009). When GFX was dissolved in less polar solvent (acetonitrile), the band shifted to red from 284 to 299 nm without much change in intensity, while the 333 nm band flattered. This effect, combined with the high extinction efficient, could be the basis for assigning the absorption bands to the $\pi\rightarrow\pi^*$ transition. The effect of pH on the absorption properties of GFX was also notable. The 284 nm band showed a small blue-shift of 6 nm when pH was increased from 2.3 to 7.4, its intensity decreased slightly. A small red-shift of 5 nm was observed when the solution became more basic (Fig. 1). The 333 nm band developed a shoulder in the basic medium, while it became flatter in acid medium. From a plot of the position of the main absorption band against pH, two pK_a values of 5.7 and 8.8 were obtained, corresponding to the pK_a, for the dissociation of the carboxylic and the 7-amino groups of the molecule, respectively. This process has been also observed in other FQs (Navaratnam and Claridge, 2000; Sortino et al., 1998;

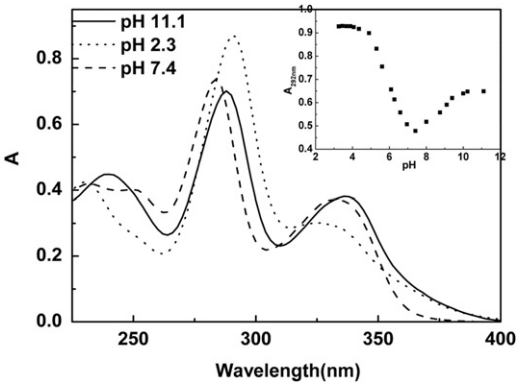

Fig. 1. UV–vis absorption spectra of GFX in aqueous solution at different pH values. The inset shows the variation of 292 nm absorption against pH values.