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Photolytic and photocatalytic decomposition of aqueous ciprofloxacin: Transformation products and residual antibacterial activity

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ABSTRACT

Previous work demonstrates that widely used fluoroquinolone antibacterial agents, including ciprofloxacin, are degraded by means of aqueous ultraviolet photolytic and titanium dioxide (TiO_2) photocatalytic (using both ultraviolet-A (UVA) and visible light (Vis) irradiation) treatment processes. In this study, we investigate the effects of photolytic and photocatalytic treatment processes on the antibacterial activity of ciprofloxacin solutions under controlled laboratory conditions. In agreement with earlier work, rates of ciprofloxacin degradation under comparable solution conditions (100 μM ciprofloxacin, 0 or 0.5 g/L TiO_2, pH 6, 25 °C) follow the trend UVA-TiO_2 > Vis-TiO_2 > UVA. Release of ammonia and fluoride ions is observed and a range of organic products have been identified with liquid chromatography–tandem mass spectrometry. However, the identified organic products all appear to retain the core quinolone structure, raising concerns about residual antibacterial potency of the treated solutions. Quantitative microbiological assays with a reference *Escherichia coli* strain indicate that the antimicrobial potency of ciprofloxacin solutions track closely with the undegraded ciprofloxacin concentration during photolytic or photocatalytic reactions. Quantitative analysis shows that for each mole of ciprofloxacin degraded, the antibacterial potency of irradiated solutions decreases by approximately one "mole" of activity relative to that of the untreated ciprofloxacin solution. This in turn indicates that the ciprofloxacin photo(cata)lytic transformation products retain negligible antibacterial activity relative to the parent compound. The energy demands for achieving one order of magnitude reduction in antibacterial activity within the experimental system are estimated to be 175 J/cm^2 (UVA-only), 29 J/cm^2 (Vis-TiO_2), and 20 J/cm^2 (UVA-TiO_2), which indicates that the UVA-TiO_2 photocatalysis is the most energy efficient process for achieving ciprofloxacin inactivation under laboratory conditions.

Abbreviations: FQ, fluoroquinolone; MIC, minimal inhibitory concentration; PEQ, potency equivalent; UVA, ultraviolet-A; Vis, visible; HPLC, high performance liquid chromatography; LC–MS/MS, liquid chromatography with tandem mass spectrometry; ESI, electrospray ionization.

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1. Introduction

Fluoroquinolones (FQs) are a class of broad-spectrum antibacterial agents widely used for both human medicine and livestock operations (WHO, 1998). Their major mode of action is inhibition of DNA replication in bacteria via interference with the normal function of two key enzymes, DNA topoisomerase II (i.e., gyrase) and DNA topoisomerase IV (Walsh, 2003). As FQs are incompletely metabolized during human therapy, a significant fraction (up to ~90%, but generally from 20 to 80%) of FQs are excreted in their pharmacologically active forms (Gerding et al., 1996), leading to their subsequent discharge into municipal sewerage. As a result of their widespread use and relatively high chemical stability, a significant load of FQs is discharged to domestic sewage (Golet et al., 2003; Watkinson et al., 2007).

Although conventional biological wastewater treatment processes can remove a significant fraction of FQs from sewage, primarily by adsorption to sludge (Golet et al., 2003), treatment is incomplete, and FQs are frequently discharged in treated secondary effluent at concentrations of ten to several hundred ng/L (Golet et al., 2003; Watkinson et al., 2007). Residual concentrations of FQs and other antibacterial agents in secondary wastewater effluent may still be harmful to organisms present in effluent-dominated receiving waters (Wilson et al., 2003). For example, recent work illustrates that mixtures of various antibacterial classes can exert unexpectedly high levels of algal growth inhibition at individual concentration levels on the order of 1 μg/L (Yang et al., 2008). Furthermore, when one considers the frequent co-occurrence of multiple FQs within wastewater matrices (Golet et al., 2003; Watkinson et al., 2007), it is clear that the total biologically effective levels derived from all such compounds may be appreciably higher than the activity attributable to a single compound.

Within a typical wastewater treatment facility, conventional wastewater treatment will result in prolonged exposure of wastewater-borne bacteria to significantly higher FQ concentrations than are present in the treated effluents described above (Golet et al., 2003; Watkinson et al., 2007), on account of the extended biomass solids retention times at which secondary clarifiers frequently operate. Such circumstances may be of particular importance for FQs such as ciprofloxacin, which has been detected by several groups within secondary wastewater influents at concentrations approaching minimal inhibitory concentrations (MICs) for various bacterial strains (Golet et al., 2003; Watkinson et al., 2007) (for a summary of relevant MICs, see (Wiedemann and Grimm, 1996)). Extended exposure of bacterial communities to MIC levels of an antibacterial compound is in turn a condition which can favor evolution of low-level antibacterial resistance in affected bacterial communities (Baquero, 2001; Drlica, 2003).

The above considerations suggest that unnecessary exposure of wastewater-borne and environmental microbiota to biologically active antibacterial compounds should be minimized whenever possible. One means of achieving this objective would be implementation of new treatment technologies capable of selectively and efficiently eliminating the biological activities of antibacterial compounds. A number of previous studies have shown that FQs are susceptible to direct photochemical transformations by exposure to ultraviolet (UV) light (Fig. 1) (Albini and Monti, 2003; Sunderland et al., 2001). Some investigations also report limited evidence that such processes can diminish the antibacterial activities of the parent compounds (Phillips et al., 1990; Sunderland et al., 2001), though the generation of biologically active intermediates has been reported for irradiation of FQs containing tertiary aliphatic piperazinyl nitrogens (e.g., ofloxacin and levofloxacin) (Sunderland et al., 2001).

Recent studies also report rapid degradation of FQs by photocatalytic mechanisms in aqueous systems containing titanium dioxide (TiO_2). UV-TiO_2 photocatalysis of flumequine is proposed to proceed by both hydroxyl and superoxide radical attack (Palominos et al., 2008). Additionally, deactivation of fluoroquinolone antimicrobial activity upon UV photocatalytic treatment has been demonstrated for some fluoroquinolones (Calza et al., 2008; Palominos et al., 2008). Paul et al. (2007) also reported on the photocatalytic transformation of ciprofloxacin and related FQs by visible and UVA wavelengths of light. Visible light photocatalysis is proposed to occur by photo-oxidation of adsorbed FQ-TiO_2 surface complexes, whereas UVA photocatalysis appears to follow both this mechanism as well as hydroxyl radical attack photoinitiated by semiconductor charge separation (Fig. 1). The visible light-TiO_2 process observed with FQs is uncommon amongst aquatic contaminants, and the FQ-selective nature of the process may be exploited to selectively treat FQs within complex aquatic matrices.

The degree to which FQs can be rendered biologically inactive (i.e., deactivated) by photochemical and photocatalytic treatment is an important criterion for determining the overall efficacy of these processes. Although several photochemical and photocatalytic transformation products of FQs have been reported, the effects of these treatment processes on the biological activity of FQ solutions have only been examined qualitatively or semi-quantitatively in a limited number of studies (Calza et al., 2008; Palominos et al., 2008; Phillips et al., 1990; Sunderland et al., 2001). With this in mind, the primary objective of the present study is to *quantitatively* assess changes in antibacterial potency of aqueous ciprofloxacin solutions during photo(cata)lytic treatment processes conducted under carefully controlled experimental conditions and correlate these changes to transformations of ciprofloxacin structure. Antibacterial activities of treated and untreated ciprofloxacin solutions were quantified using a previously reported microbiological broth microdilution assay that uses wild-type *Escherichia coli* K12 (ATCC 23716) as a reference bacterium (Dodd et al., 2009). In addition, liquid chromatography–tandem mass spectrometry analyses of irradiated ciprofloxacin solutions – supplemented by corresponding NH_3 and F^- measurements – were undertaken to facilitate identification of prominent transformation products generated under different reaction conditions. Finally, energy requirements for removing the antibacterial activities of ciprofloxacin solutions with each photo(cata)lytic process were estimated to permit comparison of the three processes' potentials as sustainable treatment strategies for waste streams containing FQs.