The photodegradation of metronidazole in the presence of coexisting pharmaceuticals

Bo Wu, Ting Zhang, Jinxia Li, Yong Ye and Huaixia Chen

ABSTRACT

The objective of this study was to investigate if coexisting compounds could affect the fate of pharmaceuticals in surface water under solar irradiation. The degradation of metronidazole (MET) in the presence of different coexisting pharmaceuticals was investigated in batch experiments with exposure to sunlight. Tinidazole, which has a similar structure to MET, was employed as an analogue. The results indicated that the presence of an analogue with a similar photosensitive group to MET could inhibit the photodegradation of MET. In addition, the effect of coexisting pharmaceuticals with different absorption spectra on the degradation of MET was investigated. The results showed that the effect depended on the degree of overlapping absorption spectra between MET and the coexisting pharmaceuticals. The relationship between the degree of the influence and the ultraviolet absorption spectra of coexisting pharmaceuticals found in this study could give guidance in assessing the fate of pharmaceuticals in environmental water.

Key words | absorption spectra, coexisting pharmaceutical, kinetics, metronidazole, photodegradation

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INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) have recently received much attention as organic micro-pollutants in aquatic environments (Kolpin *et al.* 2002; Metcalfe *et al.* 2003). A wide range of PPCPs have been detected in a variety of environmental samples at levels ranging from ng L^{-1} up to g L^{-1} (Yu *et al.* 2006; Kim *et al.* 2009). The continuous discharge of pharmaceuticals into the environment results in chronic exposure of aquatic organisms to these compounds and/or their bioactive metabolites (De Lange *et al.* 2006), because their transformation/removal rates are compensated by continuous input into the environment. The degradation products of PPCPs are increasingly common environmental pollutants (Gómez *et al.* 2008) too. So, it is necessary to investigate the transformation of these pollutants in the environment to enable accurate ecological risk assessments of PPCPs.

Photodegradation of PPCPs caused by solar irradiation is of major significance in the natural elimination process (Boreen *et al.* 2003; Tusnelda & Frimmel 2003; Robinson *et al.* 2007). Several pharmaceuticals, including tetracycline (Werner *et al.* 2006), oxytetracycline (Pouliquen *et al.* 2007), nitrofuran antibiotics (Edhlund *et al.* 2006), mefenamic acid (Werner *et al.* 2005), and β-blockers (Liu & Williams 2007) were found to undergo photodegradation under solar irradiation.

Pharmaceuticals can undergo photodegradation directly, resulting from the direct absorbance of photons, as well as indirect photodegradation initiated by light absorption by other chemicals in the system (Lam *et al.* 2003; Katagi 2004). In previous studies, many aquatic constituents such as humic acids, Cl^-, Br^-, I^-, Fe(III), NO^{3-}, and CO_3^{2-} have been demonstrated to accelerate or inhibit indirect photodegradation (Miller & Chin 2005; TerHalle & Richard 2006; Halladja *et al.* 2007; Ge *et al.* 2009a). Self-sensitized photooxidation of pharmaceuticals, e.g., tetracycline (Werner *et al.* 2006; Engel *et al.* 2008), phenicol antibiotics (Ge *et al.* 2009b), and 1-benzyl-3,4-dihydroisoquinolines (Martin & Jefford 2004), has also been reported and this can affect the photodegradation rate of PPCPs.

In fact, two or more PPCPs have been detected at the same time in a variety of environmental samples (Miao *et al.* 2004; Boxall *et al.* 2005; Hamscher *et al.* 2005; Petrovic *et al.* 2006), indicating that coexisting pharmaceuticals have become important constituents in aquatic environments.

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However, coexisting pharmaceuticals, as exotic constituents, have seldom been taken into account in assessing the photochemical fate of pharmaceuticals in the environment. We performed a literature survey and found only one relevant study. Doll & Frimmel (2003) investigated the photodegradation of pharmaceuticals in the presence of other pharmaceuticals and found that the degradation rate constants of carbamazepine in the presence of different initial concentrations of clofibric acid, which acted as a competitive inhibitor, were lower than the calculated values. To the best of our knowledge, comprehensive investigation of the photochemical behavior of pharmaceuticals in the presence of coexisting pharmaceuticals has not been reported previously.

The focus of this study was to investigate if coexisting compounds can affect the fate of pharmaceuticals in surface waters under solar irradiation. Thus, analogue and non-analogues of metronidazole were employed to assess the influence of coexisting pharmaceuticals.

METHODS

Chemicals

Metronidazole (MET), tinidazole (TNZ), enoxacin (ENO), furazolidone (FZD), ketoprofen (KPF), naproxen (NPX), amoxicillin (AMX), and diclofenac sodium (DS) of 99% purity were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Methanol and acetonitrile (HPLC grade) were obtained from Tedia Company Inc. (Ohio, USA). Ultrapure water was produced with an Ultrapure Water System (Beijing, China). Stock solutions of all test chemicals were prepared in pure water at a concentration of 2.5 mM and stored at 4 °C in a refrigerator. Working standard solutions were prepared by appropriate dilution of the stock solution using deionized water.

Hydrolysis experiments

Two solutions of MET (0.05 mM) were prepared in deionized water, adjusted to pH 4.5 and pH 7.5 using phosphate buffers, and kept in glass test tubes covered by aluminum foil. The temperature was maintained constant at 25 °C by means of a water bath. Aliquots of each sample were withdrawn at appropriate intervals and analyzed by HPLC in order to monitor the substrate decay.

Photodegradation experiments

Photodegradation of the selected pharmaceuticals in pure water under solar irradiation was evaluated. The solution with appropriate concentration of individual PPCPs was prepared by adding stock solution to each vessel, and then the solution was transfered to quartz tubes. Experiments were conducted during July and August in 2010. Quartz tubes filled with 20 mL of solution were exposed to solar irradiation on a building's terrace in Wuhan (30°58′ N, 114°33′ E). Quartz tubes containing the solutions were held approximately 45° from the horizontal, and exposed to clear sunshine from 09:00 to 16:00 h. The whole exposed time was 6 h for each batch. The light intensity (200–420 nm) in the center of the reactive solutions was 7.37 mW cm^{-2} sunlight. After irradiation, samples were withdrawn from the quartz tubes and promptly analyzed.

Quantum yield (Φ) in pure water was measured with sunlight irradiation and by using potassium ferrioxalate as a chemical actinometer (Hatchard & Parker 1956). Dark controls shielded from light were performed simultaneously under the same conditions. Photodegradation experiments and the dark controls were carried out in triplicate.

Analytical determinations

Initial and residual concentrations of the MET were analyzed with a Dionex Summit U3000 HPLC system equipped with a manual injector and a Photodiode Array Detector (Dionex Technologies, USA). An amethyst-C18 column (4.6 × 250 mm, 5 μm; Sepax Technologies Inc., Newark, NJ, USA) was connected with a guard column (cartridge 2.1 × 12.5 mm, 5 μm; Agilent Technologies, Palo Alto, CA, USA) filled with the same packing material. The mobile phase was a methanol–water mixture (35:65, v/v) and the flow rate was 1.0 mL min^{-1}. The UV detector was set at a wavelength of 320 nm for analytes. The absorption spectra (Figure 1) of pharmaceuticals were measured by an ultraviolet and visible spectrophotometer (UV-Vis2300, Tianmei Instrumental Co., China).

RESULTS AND DISCUSSION

At room temperature, the concentration of MET decreased by only 1.8% in control solutions in pure water kept in the dark for 10 days. This result indicated that the contribution of hydrolysis to decay was negligible during the photodegradation experiments. MET's wavelength-averaged

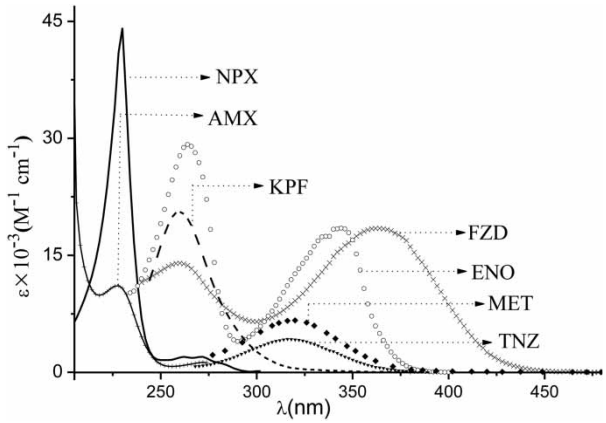


Figure 1 | Ultraviolet absorption spectra of PPCPs used in this study. All pharmaceuticals were dissolved in water.

(250–350 nm) quantum yields were determined to be 0.122 ± 0.001.

Photodegradation of MET in the presence of analogues

In order to investigate the effect of the presence of analogues on photodegradation of MET, TNZ, which has a similar absorption spectrum, to MET was selected (Figure 1). Photodegradation of MET in the presence of TNZ at different initial concentrations is shown in Figure 2. Linear relationships between $\ln(C/C_0)$ and time (t) showed that the photodegradation of MET followed pseudo-first-order kinetics ($r^2 > 0.95$). The measured degradation rate constant of MET decreased stepwise from 1.45×10^{-2} to 7.65×10^{-3} min^{-1} as the initial concentration of TNZ rose from 0 to 0.2 mM. The results suggested that the coexisting TNZ could greatly inhibit the photodegradation of MET, and the inhibition increased with increasing TNZ concentration.

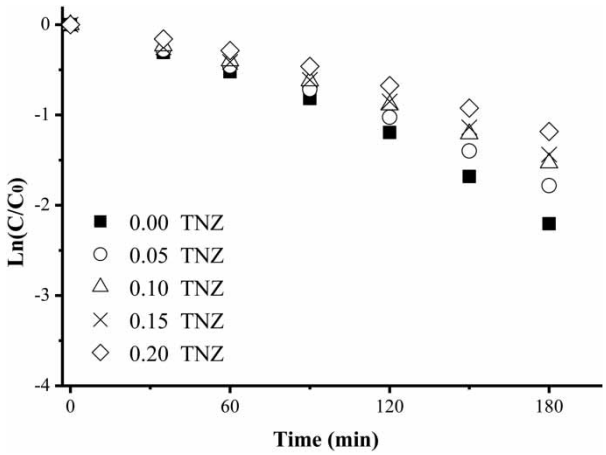


Figure 2 | Photodegradation kinetics of MET (0.20 mM) in the presence of TNZ (mM) at different initial concentration under solar irradiation.

The photodegradation rate of MET decreased with increasing initial MET concentration (Figure 3); this is thought to occur because of the complete absorption of the incident photon flux by the higher initial pharmaceutical concentrations over a shorter pathlength and light screening effects (Tusnelda & Frimmel 2003; Cogan & Haas 2008). As can be seen from Table 1, TNZ and MET have the same ring and functional groups containing nitrogen, which seem to be the sites where the photodegradation reaction of MET occurs (Dantas *et al.* 2010). Also, TNZ has similar absorption spectra to MET (Figure 1). Therefore, for the photodegradation of MET, the presence of TNZ has a similar impact to increasing MET concentration. The results suggested that the presence of an analogue, which has a similar photosensitive group and similar absorption spectra to the pharmaceutical of interest, could produce an inhibitory effect on photodegradation.

Photodegradation of MET in the presence of non-analogues

Because of its similar photosensitive group to MET, TNZ was found to inhibit the photodegradation reaction of MET. For comparison, two compounds which lack the photosensitive group were selected to investigate the effect of the presence of non-analogues: FZD with a similar absorption spectrum (350 nm) and AMX with a dissimilar absorption spectrum (230 nm) to MET (319 nm).

Photodegradation of MET in the presence of FZD or AMX with different initial concentrations was investigated. Compared with the photodegradation of MET in pure solution, obvious inhibition was observed when FZD was present (Figure 4). Photodegradation rate constants of

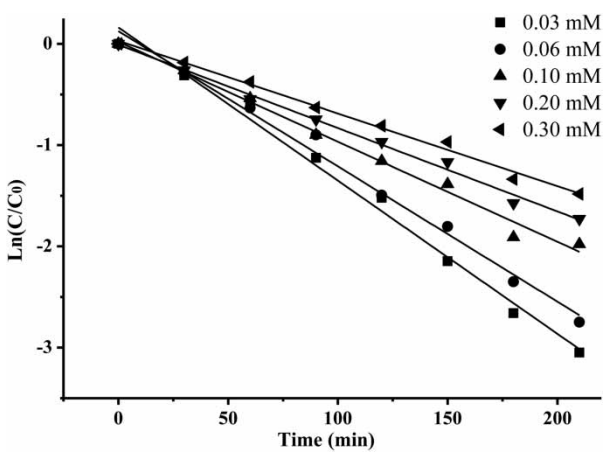
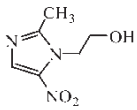
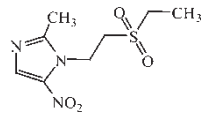
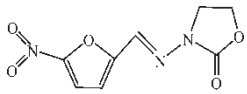
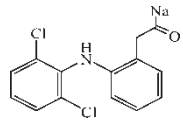
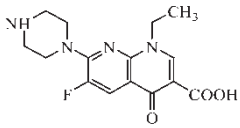
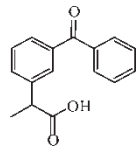
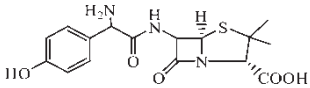
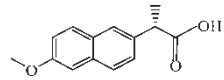


Figure 3 | Effect of initial concentration on the photodegradation of MET in pure water under solar irradiation. Initial conditions: pH 5.4, 15 °C.

Table 1 | Structures of the target PPCPs used in this study

Compound	Structure	Compound	Structure
Metronidazole		Tinidazole	
Furazolidone		Diclofenac sodium	
Enoxacin		Kepotrofen	
Amoxicillin		Naproxen	

MET were reduced from 1.45×10^{-2} to 9.89×10^{-3} min^{-1} in the presence of 0.2 mM FZD. Like the effect of an analogue (TNZ), the reaction rate of MET decreased with increasing FZD.

In contrast, the presence of AMX has no effect on the photodegradation of MET (Figure 5). One likely reason is the lack of overlap in absorption spectra of AMX and MET (Figure 1). So the inhibition of FZD on the degradation of MET was probably related to the similar ultraviolet absorption spectrum between FZD and MET. Doll & Frimmel (2003) found that the presence of clofibric acid causes competitive inhibition (an inner filter) on the photochemical degradation of carbamazepine. The absorbed photon fluxes by carbamazepine and clofibric acid in the irradiated samples of the UV solar simulator were calculated from the measured data of irradiance and absorbance, taking into account the competitive absorption of both components. The comparison of calculated and measured degradation rates showed that the competitive inhibition was not only an inner filter effect. There must also be a scavenging of reactive intermediates and/or a stabilization of the compounds by formation of complexes.

The dependence of the degradation rate constants on the ultraviolet absorption spectra of coexisting pharmaceuticals shows that the degradation of the pharmaceuticals could be affected by the coexisting pharmaceuticals with a similar absorption spectrum.

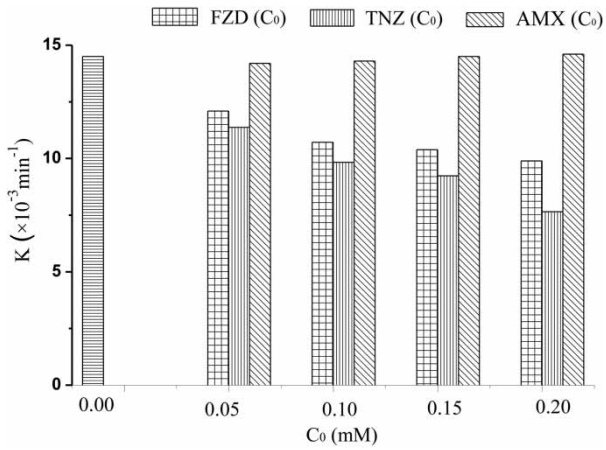


Figure 5 | The photodegradation rate constants of MET (0.20 mM) in the presence of FZD, AMX, or TNZ with different initial concentration.

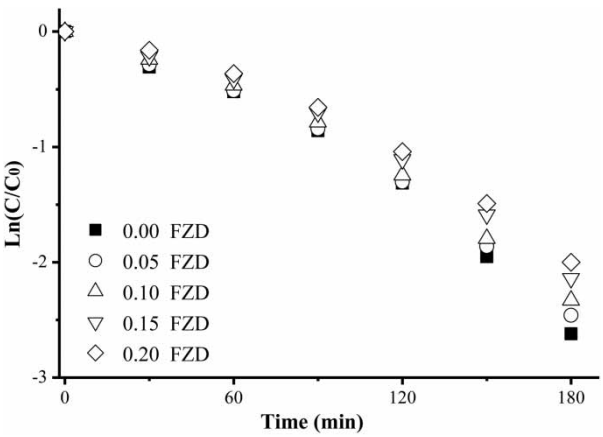


Figure 4 | Photodegradation kinetics of MET (0.20 mM) in the presence of FZD (mM) with different initial concentration under solar irradiation.

Molar absorption vs photodegradation

As it was shown that coexisting pharmaceuticals, such as FZD, which have similar ultraviolet absorption spectra to MET could inhibit the photodegradation of MET, the relation between the influence on the photodegradation and the absorption spectra of coexisting pharmaceuticals was investigated. AMX, KPF, NPX, DS, FZD, and ENO were selected according to their overlapping degree of the absorption spectra with MET (Figure 1).

The degradation rate constants of MET in the presence of different pharmaceuticals is shown in Figure 6. By comparing absorption spectra, we conclude that the compounds which inhibited the photodegradation of MET are those whose absorption spectra overlap, more or less, with MET's. In general, MET exhibited smaller k values in the presence of pharmaceuticals with higher UV absorbance at 260–350 nm (except for TNZ). Coexisting pharmaceuticals such as ENO and FZD, which have more overlap with MET in absorption spectra, could affect the photodegradation of MET more strongly. In contrast, the influence of coexisting pharmaceuticals such as KPF and AMX, which have less or no overlap with MET, was negligible. These observations indicated that the influence of the coexisting pharmaceuticals on the degradation of MET was partly determined by the degree of overlapping absorption spectrum between the coexisting pharmaceutical and MET.

Molar absorption coefficient, which is a measure of absorbed photon energy, is one important parameter in photodegradation of pharmaceuticals (Giri *et al.* 2010). In addition, the molar absorption coefficient of pharmaceuticals in the presence of other coexisting organic compounds can be expected to vary greatly due to variation in physicochemical characteristics of the compounds and limitations of available photon energy.

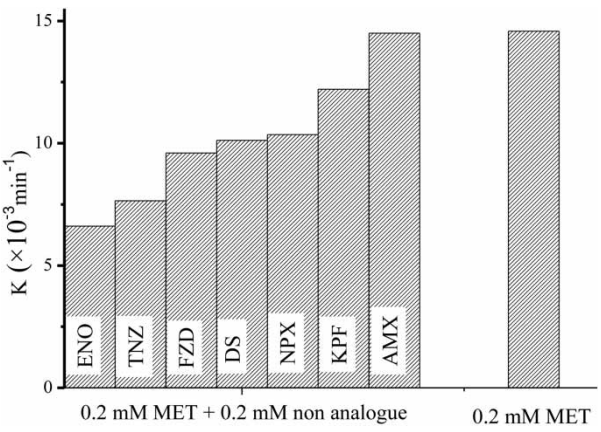


Figure 6 | The photodegradation rate constants of MET in the presence of different non-analogues.

CONCLUSION

This study showed that the coexisting pharmaceuticals, both analogue and non-analogue, are likely to inhibit the photodegradation of MET, and the inhibition was greater with the increase of the initial concentration of coexisting pharmaceuticals. Further study demonstrated that coexisting pharmaceuticals which have similar absorption spectra could affect the photodegradation reactions of MET. The influence was determined by the degree of overlapping absorption spectra between the coexisting pharmaceuticals and MET. Compared with other organics and metals typically occurring in natural surface waters, such as humics and bicarbonate ions, the effect of analogue pharmaceuticals is expected to be significant. This observation shows that coexisting pharmaceuticals are indissolubly connected with the fate of pharmaceuticals undergoing photodegradation in aquatic environments and, should be taken into account in assessing the photodegradation of pharmaceuticals.

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