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Photo-Degradation of Amoxicillin, Streptomycin, Erythromycin and Ciprofloxacin by UV and UV/TiO_2 Processes. Evaluation of Toxicity Changes Using a Respirometric Biosensor

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Abstract

Current research aims to conserve natural resources, especially water. Our study focuses on developing water purification to remove contaminating antibiotics using heterogeneous photo-catalysis. We compare photolysis with and without TiO2 catalysis, testing the photo-stability of four representative antibiotics: amoxicillin, streptomycin, erythromycin and ciprofloxacin by HPLC using a Perkin Elmer 2000 chromatograph, BIO-RAD Bio-sil ODS-5S L 250 mm × 4 mm ID C18 reversed phase column and Perkin Elmer LC-90 UV detector. Both obeyed first order reaction kinetics, usually following the Lagmuir-Hinshelwood model. A respirometric biosensor was employed to assess the toxicity of drug solutions and photo-degradation products. We demonstrate that catalysis consistently decreases toxicity more effectively in all cases.

Keywords: Amoxicillin; Streptomycin; Erythromycin; Ciprofloxacin; Photo-catalysis; UV/TiO_2; Respirometric biosensor

Introduction

Recently, drug residues contaminating water supplies and their ecological effects [1] has attracted scientific attention. These molecules are intended to induce useful physiological effects for diagnosing, treating or preventing diseases but have significant environmental and sanitary impacts. Partially metabolised by host enzymes, both unchanged antibiotics and pharmacologically active metabolites are excreted in urine and faeces [2,3]. Sewage treatment plants (STP) consequently receive large quantities of active drugs in wastewater, they are often incompletely removed, releasing significant quantities to the environment. Due to the widespread use and limited biodegradability of antibiotics, they are ubiquitous [4-6], persisting dependent on their chemical/physical characteristics. Erythromycin has a ½-life >1 year. Environmental drug concentrations range between nano- and micro-grams/litre, posing no risk of acute toxicity but possible dangers from chronic exposure [7]. Toxic effects depend on: dose, exposure duration, administrative route and characteristics of the exposed organism, their action on human subjects isn't the only problem, environmental antibiotics can induce bacterial resistance [8,9], posing dangers to public health. Increasingly, infectious bacteria become resistant to available remedies. We seek to propose a photo-catalytic method to enhance water treatment and reduce pollutants to acceptable levels. Photolysis and photo-catalysis are compared and a photo-chemical method for removing antibiotics from water developed.

Materials and Methods

Materials

Chemicals: Representatives of four classes of water-soluble antibiotics frequently contaminating European water supplies [10-12] were chosen and photo-degraded. Amoxicillin is a β-lactam, streptomycin sulphate an aminoglycoside, erythromycin a macrolide and ciprofloxacin a fluoroquinolone. Distilled water was used throughout. Amoxicillin was extracted from 1 g Velamox trihydrate tablets, ciprofloxacin from Teva; Applichem supplied erithromycin and streptomycin sulphate.

Preparation of solutions: Water Samples were weighed with an analytical balance and dissolved in distilled in volumetric flasks.

The procedure adopted for tablets was:

1. Grind finely and homogenously in a porcelain mortar.

2. Decant into centrifuge tube, with ~ 8 mL distilled water.

3. Sonicate for 5 minutes.

4. Centrifuge at 10,000 rpm for 10 minutes.

5. Transfer supernatant using a pipette to a flask.

6. Add a further 8 mL of solvent, sonicate for 5 minutes, centrifuge and repeat step 5.

7. Repeat step 6 until all was dissolved.

8. Transfer powder to a funnel covered with filter paper and wash down.

9. Transfer rinse water to flask in step 5 and bring to volume.

10. Analyze resulting solution by spectrophotometry and HPLC.

Instruments

Uv-vis: Samples of drug solutions were examined using a Perkin-Elmer Lambda 16 dual-beam spectrometer.

We used appropriate concentration and measured the maximum absorption wavelength, λ max, to set the HPLChromatograph UV detector.

HPLC: Using a Perkin Helmer 2000 chromatograph and BIO-RAD Bio-sil ODS-5S L 250 mm × 4 mm ID Perkin Elmer LC-90 UV detector, analyses was performed at room temperature under isocratic conditions.

The compositions and acidity were:

For amoxicillin [13]: pH 6 phosphate buffer: methanol (95:5) at 0.8 mL/min.

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For erythromycin [14]: 0.02M potassium phosphate dibasic buffer: acetonitrile (60:40) at 1 mL/min.

FM streptomycin [15,16] : pH 3.3 water+2 mL of phosphoric acid: acetonitrile (80:20)

For ciprofloxacin [17]: pH 3, 0.042 mM aqueous phosphoric acid: acetonitrile (87:13) at 0.8 mL/min.

We followed progress using chromatograms of photo-degradation at set times by comparing the initial and final chromatographic peak areas with an HPLC UV-VIS data-logger (DrDAQ) recording analogue voltages. The Pico Log software employed processed the data on a PC and Peak Fit v 4.12 calculated the areas.

Methods

Photolysis and photo-catalysis: Photo-degradation, usually by sun light energy, forms highly reactive chemicals such as OH radicals [18,19] promoting substrate degradation [20]. Interactions between light and organic molecules in aqueous solution lead to photolysis. Photon absorption induces electron transfer to excited states breaking bonds and changing the molecular structure:

$$\text{substrate} + h\nu \rightarrow \text{degradation products } CO_2 + H_2O + \text{inorganic salts}$$

Using direct photolysis for photo-degradation is cheaper but less efficient than combining light with oxidizing agents. Heterogeneous photo-catalysis is oxidative, light enables solid semiconductors to catalyze photo-degradation. The positive aspects of this technology are its effectiveness on recalcitrant molecules, speed and cost.

The literature reports using semiconductors ZnO, CeO_2, CdS, ZnS, etc as catalysts [21,22], the most efficient and commonly used semiconductor being titanium dioxide TiO_2 [22-26] occurring naturally as: anatase, rutile and brookite, it's photo-stable, insoluble and non-toxic [26,27]. We used anastase since several reports identify it as the best crystalline form [18,28]. A Lightning Cure LC8 Hamamatsu 9566-02 L UV lamp served to photo-degrade the drug solutions at wavelengths 240-400 nm. An optic fibre cable emitting UV light was inserted in a plywood structure housing a magnetic stirrer. Parallel TiO_2 (DEGUSSA P25) catalyzed photolytic degradations served as controls. They were irradiated in 50 ISO/GL Duran glass bottles and 0.01 g TiO_2 was added to assess the photo-stability of 20 mL samples as described above. Fractions awaiting analysis were kept in the dark to avoid photo-degradation. Catalytic degradation products were centrifuged at 10,000 rpm for 14 min, supernatants collected with Pasteur pipettes and centrifuged for 7 min at 10,000 rpm and analyzed.

Toxicity tests: We intend to demonstrate that catalysed photo-degradation transforms toxic drugs to safer degradation products. Toxicity was evaluated by measuring inhibition of Saccharomyces cerevisiae [29-31] respiration.

Principle

Breathing enables biochemical combustion of nutrients, providing aerobic cells with energy for fuelling vital functions [32], e.g. combustion of glucose is caytalyzed:

$$C_6H_{12}O_2 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$

Yeast respiration is sensitive to toxins, Clark amperometric electrodes Shown in figure 1 comprising an electrolytic cell with Pt/Au cathodes and Ag/AgCl anodes separated by epoxy resin determine [O_2], enabling evaluation of this inhibition. The electrodes on plastic supports are immersed in the electrolyte, behind a Teflon gas-permeable membrane, using a potential ~ -650 mV w.r.t the anode. Figure 1 At the cathode, O_2 is reduced:

$$O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O, \text{ whilst at the anode}: Ag^+ + Cl^- \rightarrow AgCl + e^-$$

The current between cathode and anode is proportionate to the oxygen level outside the membrane. Yeast plates were prepared following Campanella et al. [30]. Culture plates were stored in a refrigerator upside down wrapped in parafilm for up to 15 days (before becoming inactive). A diskette tablet with diameter matching the electrode tip was etched from a plate and fixed outside the Clark electrode's Teflon membrane, through a dialysis membrane and O-ring. This biosensor was immersed in 10 mL of distilled water at 25°C in a 50 mL beaker with a magnetic stirrer. 10 mL of 1M glucose was added when the electrode measuring ppm O_2 without yeast respiration was stable. Glucose triggers yeast respiration, consuming oxygen and reducing the signal, (Δppm O_2) B, when stable analysis was complete. After incubating the tablet for 3 hours, toxicity was assessed using the same process, it inhibited respiration, giving signal (Δppm O_2)T. Plotting [O_2] vs time yields curves resembling those in Figure 2.

Results

Spectrophotometry

Table 1 lists the Pelkin-Elmer Lambda 16 spectrometer readings from which the drug concentrations were chosen, [11,12] report their solubility.

Photo-degradation

Figures 3 and 4 show chromatograms of amoxicillin degradation by photolysis and by TiO_2-catalyzed photo-degradation, they illustrate its sensitivity to the latter. After 30 min exposure to UV rays in the presence of catalyst is completely mineralized. Ciprofloxacin shows another trend, Figure 5 shows how drug concentration decreases with photolysis with different irradiation times and additional peaks due to degradation product formation. Active drug concentrations decrease rapidly with photo-catalysis, the by-products quickly disappear as irradiation progresses. Ciprofloxacin exposed to UV radiation, photolysis tests find rapidly decreasing drug concentrations, and after 30 minutes' irradiation it's almost completely photo-degraded. After 1 minute's photo-catalysis, ciprofloxacin is almost completely degraded. Figure 6 shows results of photo-stability tests illustrating the The most

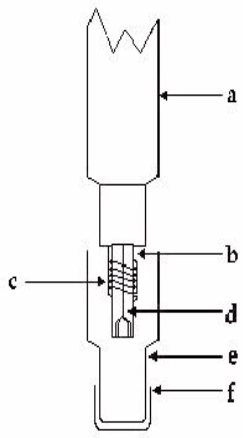

Figure 1: Scheme of a Clarck-type electrode: **a)** electrode body; **b)** dielectric; **c)** Ag/AgCl-anode; **d)** Pt-cathod; **e)** teflon housing; **f)** semipermeable membrane.

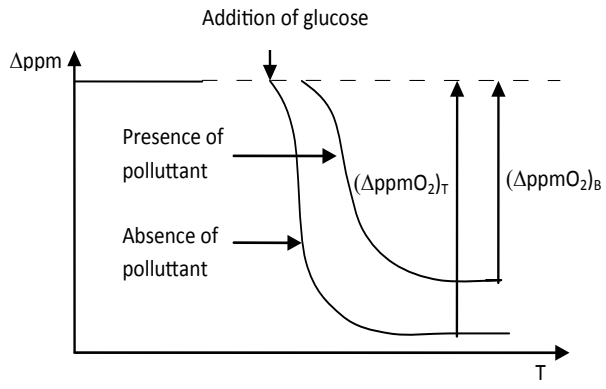

Figure 2: Trend of the oxygen concentration over time, in the absence and in the presence of pollutant.

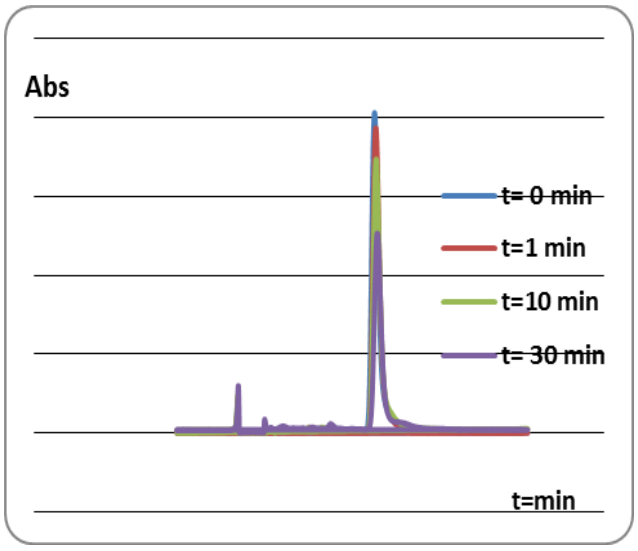

Figure 3: Photolysis of a solution of amoxicillin 10 mg/L at different time of irradiation.

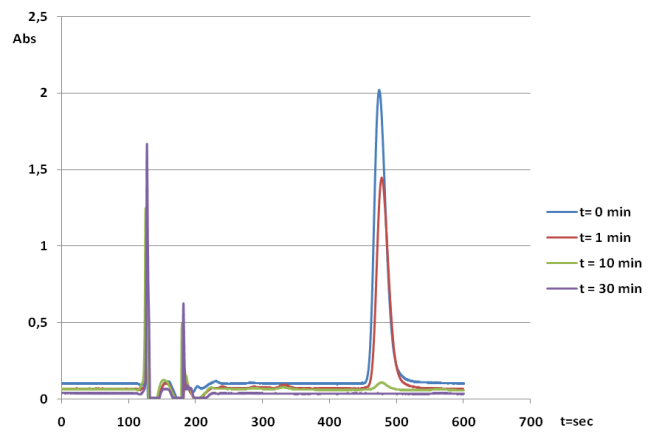

Figure 4: Photocatalysis of a solution of amoxicillin 10 mg/L at different time of irradiation with the addiction of 0.01g TiO_2.

Antibiotic	λ max	Concentration	Water sol. T=25°C
Amoxicillin	195 nm	10 mg/L	4 × 10^3 mg/L
Streptomycin sulphate	194 nm	50 mg/L	50 × 10^3 mg/L
Erythromycin	192 nm	300 mg/L	2 × 10^3 mg/L
Ciprofloxacin	275 nm	3.75 mg/L	0.065 × 10^3 mg/L

Table 1: λ-max, concentration of the solutions used and water solubility.

Antibiotic	K (photolysis)	$T_{1/2}$ (photolysis)	K (photocatalysis)	$T_{1/2}$ (photocatalysis)
Amoxicillin	0.0037 min^{-1} (±0.001)	186.2 min	0.433 min^{-1} (±0.01)	1.6 min
Streptomycin sulphate	0.017 min^{-1} (±0.01)	40.8 min	0.15 min^{-1} (±0.02)	4.55 min
Erythromycin	0.0159 min^{-1} (±0.01)	43.49 min	0.038 min^{-1} (±0.002)	18.18 min
Ciprofloxacin	0.107 min^{-1} (±0.03)	6.47 min	3,187 min^{-1} (±0.03)	0.22 min

Table 2: K: Kinetic constants calcolated by the model of Lagmuir-Hinshelwood.

Drug	IAR% ± σ Drug	IAR% ± σ photocatalysis product T=30 min	IAR% ± σ photocatalysis product T=15 h
Amoxicillin	(7.05 ± 0.06)%	(46.25 ± 0.5)%	(0.40 ± 0.01)%
Streptomycin	(56.21 ± 1)%	(7.6 ± 0.7)%	(0.10 ± 0.02)%
Erytromycin	(80.31 ± 2)%	(5.3 ± 0.9)%	(0.20 ± 0.03)%
Ciprofloxacin	(54.02 ± 1.1)%	(89.05 ± 1.1)%	(0.10 ± 0.01)%

Table 3: The results of the toxicity tests are shown in the chart.

relevant results of the photostability tests for the four drugs are show in figure 6. It is evident that the difference between photolysis (blue) and photocatalysis (red) is outstanding in the case of Amoxicillin and Streptomycin. Formulating a kinetic model for heterogeneous photo-catalysis reactions is complex; multiple reactions are possible subject to many parameters [25,33]. Adsorption need be considered in addition to degradation reactions and account taken of the unknown photo-degradation mechanism. Both photolysis and photo-catalysis degradation are first order kinetic reactions; often the Lagmuir-Hinshelwood model [16,18,34] accounts for our results.

$$ln\ C/C_0 = -k \times t$$

Where C is drug concentration at a particular reaction time and C_0 the initial concentration.

The kinetic constant k in a first order reaction is obtained by plotting - ln C/C_0 against time, its gradient giving the rate constant, K. Table 2 and Table 3 reports its value for every antibiotic subject to photolysis and photo-catalysis

Toxicity tests

Photo-degradation reduces both active principle concentrations and toxicity. We evaluate the percentage respiratory activity inhibition (IAR) generated from the pollutant relative to a blank, calculated using:

$$IAR\% = \{1 - (\Delta \text{ ppm O2})T\ (\Delta \text{ ppm O2})_B\}100$$

where: (Δ ppm O2)T = oxygen consumption when pollutant is present, corresponding to its inhibitory activity, (Δ ppm O2) B = oxygen consumption when pollutant is absent, corresponding to the blank.

Repeated respirometric observations were notably more reproducible when tablet thickness was controlled. It determines

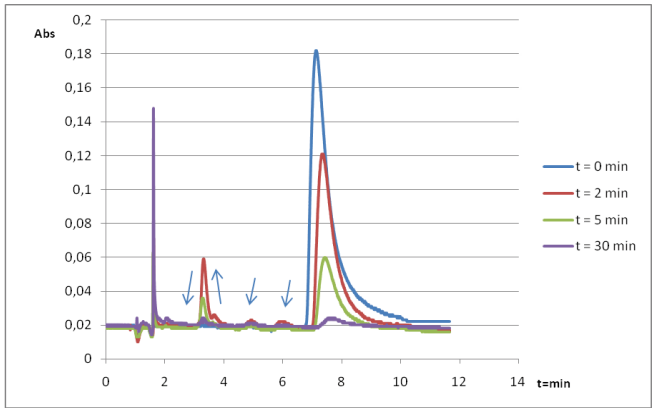

Figure 5: Photolysis of a solution of ciprofloxacin 50 mg/L at different time of irradiation.

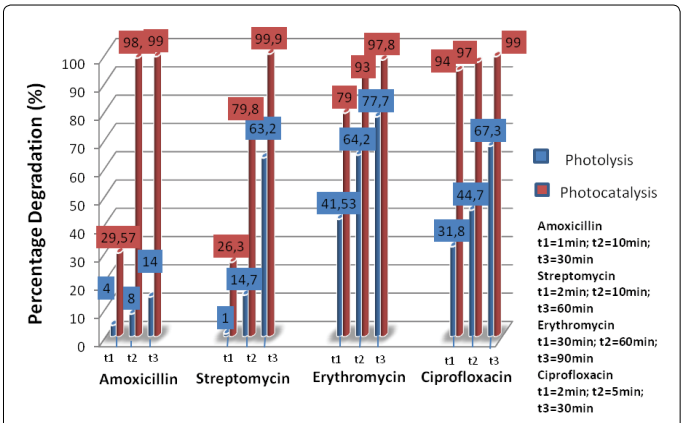

Figure 6: Histograms showing the percentage of reduction of the drugs: the blue bars indicate the percentage of degradation by photolysis, while red bars indicate the percentages of photo-degradation with titanium dioxide.

the amount of yeast collected, influencing oxygen consumption and creating a jump in the respirometric curve. This standard deviation of 1-2% ensures repeatability.

Table 3 compares the IAR values of solutions degraded in presence of TiO2 for 30 min and 15 h with those obtained by exposing yeast to solutions of the original molecules following incubation for ~ 3 hours.

Longer degradation times were needed for ciprofloxacin and amoxicillin to reduce toxicity to yeasts due to formation of toxic intermediates.

Table 3 Results of toxicity tests. IAR (inhibition of respiratory activity) of solutions of starting drugs and those exposed to TiO2 catalyzed photo-degradation for 30 min and 15 h after three hours incubation.

Conclusion

- Ciprofloxacin is most easily photo-degraded, followed by amoxicillin, streptomycin and erythromycin, the antibiotic most resistant to the experimental conditions.

- In all cases, heterogeneous photo-catalysis is demonstrated to be a powerful way to remove drugs from water and more effective than photolysis.

- Photo-degradation reaction products are shown to be less toxic than the original drugs, in certain cases longer reaction times are needed.

- Our research shows that complete drug abatement needs a combination of methods, emerging technologies need to be assessed.

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