Research article

Assessing the biodegradability of common pharmaceutical products (PPs) on the Zambian market

James Nyirenda a,*, Alexina Mwanza a,1, Chilufya Lengwe a

a School of Natural Sciences, Department of Chemistry, University of Zambia, Zambia

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ABSTRACT

Biodegradation is the breakdown of complex organic compounds into simpler molecules like carbon dioxide and water by microorganisms like bacteria and fungi. Biodegradation studies of pharmaceuticals are initially done to assess which pharmaceuticals are persistent in the environment. Whole pharmaceuticals or their metabolites are excreted from the human body via urine or fecal matter after administration. These go into the Wastewater Treatment Plants (WWTP) and are later released into the environment with the treated wastewater. Recent studies have reported a number of pharmaceuticals in the ecosystem and the effects of these on non-target species has become an issue of environmental concern. The biodegradation studies of eight pharmaceuticals were carried out in this research. The choice of pharmaceuticals was based on the most commonly prescribed medications at the University of Zambia (UNZA) Clinic in seven therapeutic groups: anti-hypertensives, antibiotic, antimalarial drugs, anti-tuberculosis, antihelminthics, antifungals and antiretroviral drugs. The biodegradability tests were carried out using a modified carbon dioxide evolution method (modified Sturm test). The inoculum was derived from the secondary effluent of the UNZA WWTP plant and Dextrose monohydrate was used as a system control. Using this guideline, the system control, dextrose monohydrate biodegraded 77 ± 0.270% in seven days. All the pharmaceuticals except ciprofloxacin were found to be non-biodegradable: Atenolol degraded 6.8 ± 0.026%, ketoconazole degraded 1.0 ± 0.003%, isoniazid/rifampicin degraded 0.8 ± 0.003%, mebendazole degraded 13.0 ± 0.050%, nevirapine degraded 1.3 ± 0.005%, pen-v degraded 1.0 ± 0.004% and quinine sulfate degraded 1.8 ± 0.008%. Ciprofloxacin showed a negative carbon dioxide evolution and it was noted that bacteria were not viable as the drug proved to be very potent against bacterial strains in the inoculum used.

1. Introduction

Pharmaceuticals and related products have become chemicals of emerging environmental concern in recent years (Xu et al., 2017). Pharmaceuticals have been used as human medicines to treat or prevent microbial infections, relieve pain, correct malfunctions of the body caused by stress, improper diet, mutations and as veterinary drugs and husbandry growth promoters in aquaculture and livestock operations among others.

The use of pharmaceutical products (PPs) has seen a sharp rise globally, with antibiotics recording an increase in consumption of 30% from approximately 50 billion to 70 billion standard units between 2000 and 2010 (The center for disease dynamics, 2015). After having a curing effect, PPs are excreted through urine and feces as a mixture of metabolites or as unchanged substances depending on the pharmacology of the substance in question into the sewage treatment plants (STP). After treatment in the STP, waste water is let out into rivers and lakes where they are exposed to sunlight and can be degraded abiotically by the sun’s UV rays. However, studies show that most of the active ingredients found in commonly used PPs do not degrade easily (Ternes, 2002) and end up accumulating in the environment as pollutants and may even end up in the drinking water. Small concentrations of some drugs like carbamazepine have been detected in drinking water (Halling-sorensen and Nielsen, 1997). Pharmaceutical companies have also been known to discharge water contaminated by biologically active compounds intentionally or unintentionally into rivers and streams.

The effects of these pharmaceuticals on the environment is currently an issue of concern (Halling-sorensen and Nielsen, 1997).

Though Biodegradation experiments have been carried out before in many developed countries like Germany, the United States (Ledin et al.,

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2010) and in some third world countries like Kenya (Vankova, 2010), they have never before been carried out in Zambia. But as the number of PPs flooding the Zambian market increases, there is need for research to be carried out in this area. Exposure of microorganisms to these persistent pollutants may lead to development of resistant strains which would need stronger therapeutic drugs and in turn cause more pollution of the environment. The absence of recorded data on this issue prompted for this research to be carried out. The fate of pharmaceuticals in the environment differs dramatically depending on the stability of the active ingredients when exposed to air, water, light and its interaction with microorganisms. For example Aspirin is biodegradable and is mineralized to carbon dioxide while Clofibrate is persistent in the environment and non-biodegradable (Richardson, 2007; Richardson and Bowron, 1985) and (Stan et al., 1994). More than 80 PPs and several metabolites have been detected in μg/L in the sewage effluent and in surface waters downstream from various WWTP (Heberer, 2002).

A study done by Ngumba et al. in Lusaka, Zambia (Ngumba et al., 2020) showed that sulfamethoxazole was present in the influent and effluent waters in the mean concentration of 33 300 ± 1 890 ng/L and 30 040 ± 3 420 ng/L, respectively. Some antiretrovirals detected were lamivudine, 49 700 ± 4 000 ng/L while zidovudine, 9 670 ± 1 290 ng/L and nevirapine with 220 ± 30 ng/L respectively. The study by Ngumba et al. revealed that various pharmaceutical products could be detected in intact form days or weeks after their release in the environment. The convenience method of sampling was used; the drugs were selected based on information collected from the University of Zambia Clinic administration. According to the clinic’s records the drugs used in this research are the most commonly prescribed medicines at that clinic hence their choice to make a sample population.

2. Materials and methods

2.1. Description of sample collection and preparation

Samples were prepared according to the recommended method 301b of the OECD test methods for read biodegradability (OECD, 1992).

All drugs in tablet form were prepared according to the guidelines provided for by the GPHF minilab manual (Jähnke and Dwornik, 2008) for preparing powdered samples. Briefly, each tablet was carefully wrapped in a clean sheet of aluminium foil and pulverized with a pestle to a very fine powder. An amount equivalent to 50 mg/L was used in each case for the test sample. Active pharmaceutical ingredients (APIs) for each drug were confirmed by a minilab TLC test according to the methods of test (Jähnke and Dwornik, 2008) and read under UV at 254 nm (Yu et al., 2016).

2.2. Preparation of mineral media

All media and working stocks were made by following the (OECD, 1992) guidelines. Potassium dihydrogen orthophosphate (KH₂PO₄), dipotassium hydrogen orthophosphate (K₂HPO₄), sodium hydrogen orthophosphate dehydrate (Na₂HPO₄·2H₂O), ammonium chloride, (NH₄Cl) and Glucose monohydrate were purchased from Sigma Aldrich, Germany. Analytical grade Calcium chloride, anhydrous, (CaCl₂), magnesium sulfate heptahydrate (MgSO₄·7H₂O) and iron (III) chloride hexahydrate, (FeCl₃·6H₂O) were purchased from HIMEDIA, India. Barium hydroxide (Ba(OH)₂), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were a kind gift from Analytical Services Consultancy Laboratory, University of Zambia. All drugs tested were a free gift from the University of Zambia Clinic. Standard drugs were obtained from the Global Pharma Health Fund (GPHF), a charity initiated and sponsored by Merck Darmstadt, Germany who supplied the GPHF-minilab (Jähnke and Dwornik, 2008). All solvents and HPTLC plates (50 mm × 100 mm) for chromatography were analytical grade and were purchased from Merck Biosciences, Germany.

2.3. Inoculum

The inoculum used was sludge obtained from the University of Zambia (UNZA) domestic sewage treatment plant. A fresh sample was collected from the aeration tank immediately before use, coarse particles were removed by sieving and 0.5 mL of the solution was used.

2.4. Procedure

A custom made air supply system with tandem carbon dioxide scrubbers was designed ab initio. Three, super saturated barium traps and seven sodium hydroxide traps each at 2.0 M concentration were set up in tandem as a double-check scrubbing system. The post sample carbon dioxide traps were set up at 0.01 M sodium hydroxide. Air was purged through the system at a rate of 75 ± 0.5 mL/min. The CO₂ free air was pumped into the 1 L conical flask containing 500 mL of mineral media and 0.5 mL inoculum. For the first three days, the test vessel only contained the mineral media and bacterial inoculum only, to precondition the inoculum to increase the precision of the results obtained.

After three days, the test substance was added to give a concentration of 50 mg/L with the addition being done according to the solubility of the test chemical. Concurrently, the standard and blank were set up according to recommendations.

Sampling was done according to the OECD guidelines (OECD, 1992) with modifications. The amount of CO₂ produced was calculated from the amount of base remaining in the absorption bottle that reacts with the HCl according to the reaction:

\[ \text{NaOH(aq)} + \text{HCl(aq)} \rightarrow \text{NaCl(aq)} + \text{H}_2\text{O(l)} \]

Part of the base reacted with the CO₂ according to the reaction:

\[ \text{CO}_2(g) + 2\text{NaOH(aq)} \rightarrow \text{Na}_2\text{CO}_3(aq) + \text{H}_2\text{O} \]

After the titrations, biodegradation was calculated as percent theoretical Carbon Dioxide (% Theoretical CO₂) according to OECD guidelines (OECD, 1992).

3. Results and discussion

Figure 1 shows the HPTLC confirmation of active pharmaceutical ingredients (API). All drugs used had to pass the 80% threshold of API.

3.1. Standard dextrose (glucose monohydrate) degradation

Dextrose was tested as a system control compound on the degradation potential. Corrected for the blank, it degraded 77 ± 0.27% in 7 days using the experimental set-up described in methods. The set-up was considered functional and no change was introduced. The lag phase was reduced by adapting the bacteria to the experimental conditions for three days before introducing the carbon source (Figure 2).

3.2. %ThCO₂ at 14 and 28 day window period

Percent carbon dioxide released was studied for 14 days and 28 days respectively according to the OECD guidelines (OECD, 1992). Information on the selected drugs was sourced according to Wishart et al. (2018). Chemical structures were modified using the chemdraw software version 12 by calling each drug name online and modification.

Table 1: shows the chemical formulae and other physical properties of the sampled drugs including their level of degradation. Ciprofloxacin, nevirapine, pen-v quinine sulfate and rifamate were tested for 14 days while Atenolol, ketoconazole, and mebendazole were tested for 28 days. Information on the structures was obtained from (Wishart et al., 2018)
3.3. Biodegradation profile for the 14-day window period

Figure 3 presents pharmaceutical drugs which were assayed for only 14 days as they had reached a plateau after three consecutive titrations.

3.4. Biodegradation profile for the 28-day window period

Figure 4 presents pharmaceutical drugs which were assayed for only 28 days which is the maximum test period for samples regardless of whether a plateau been reached or not.

The compounds under investigation were eight therapeutics from seven different groups namely antibiotics, antifungal, antihelmintic, antihypertensives, antituberculosis, antimalarial and antiretroviral. Antituberculosis drugs were treated as a separate group from the rest of the antibiotics because of their specific use in treating tuberculosis infections, unlike the other two broad spectrum antibiotics ciprofloxacin and phenoxymethylpenicillin (Pen-V) which are used to treat a wide range of gram positive and gram negative infections.

Figure 1. Shows the HPTLC F254, Aluminum support, Silica plates irradiated at 254nm UV. Panel a; Atenolol-100 mg, b; Ciprofloxacin-500 mg, c; Ketoconazole-2%, d; Mebendazole-500 mg, e; Nevirapine-600 mg, f; Penicillin V-250 mg, g; Rifamate (Isoniazid-75 mg, Rifampicin-150 mg combination), h; Quinine sulfate-300 mg. Lanes 1 and 4 for each TLC plate are for the 100% and 80% standard. Lanes 2 and 3 are for 100% sample except for panel g; where lanes 1 and 4 were 100% isoniazid and 100% rifampicin respectively. lane 2 was the combination therapy Rifamate (Isoniazid-Rifampicin combination) while lane 3 was single therapy Isoniazid. The red triangles shows the API bands and the black triangles shows the solvent front. All standards used were of analytical grade (GHFP Minilab). The Atenolol plate was developed using solid iodine crystals as a source of iodine vapor. Images were obtained by use of a Samsung Galaxy FAME GT-S6810P smartphone (5 Megapixels camera) mounted on the 3D printed blackbox.

Figure 2. Shows the degradation profile of glucose monohydrate. Values were expressed as percent means of biodegradation (% ThCO2 ± RSD). Glucose monohydrate was considered to be readily biodegradable as more than 60% degraded in less than 7 days. The graph was plotted using Graphpad prism version 6 software.

Figure 2. Figure 2 shows the degradation profile of glucose monohydrate. Values were expressed as percent means of biodegradation (% ThCO2 ± RSD). Glucose monohydrate was considered to be readily biodegradable as more than 60% degraded in less than 7 days. The graph was plotted using Graphpad prism version 6 software.

and modified by ChemDraw version 12.0 CambridgeSoft, www.cambridgesoft.com. Data was expressed as percent degradation with respect to carbon dioxide evolution.
A total of eight pharmaceuticals were tested for biodegradation using the Carbon dioxide evolution method according to the OECD guidelines of testing for biodegradability (OECD, 1992). None of the tested pharmaceuticals were readily biodegradable. The concentration of the test pharmaceuticals in the inoculum was 50 mg/L of the active ingredient except for the antibiotics and anti-tuberculosis drugs which showed an inhibitory effect on the bacteria at these high concentrations. Therefore, tests were repeated with 15 mg/L of the test pharmaceuticals. A relatively high concentration was chosen to increase the accuracy of the test. A fresh stock of the inoculum from the same source was obtained for each test and a measured volume of 0.5 mL was used. The biodegradation was calculated as % ThCO2 which was calculated from the amount of carbon dioxide produced by the inoculum. The amount of CO2 produced was determined by titrations which were done in triplicate to increase the precision of the results and the standard deviation was calculated for each set of values. The percent uncertainties of the biodegradation results were also calculated by executing the STDEV.P function in Excel version 2016. The excel data was fed into Prism version 6.05 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

The majority of antimicrobials have high EC50 values against Daphnia, bacteria and algae (Boxall, 2006, Halling-sorensen and Nielsen, 1997), which make them non-biodegradable and leads to

### Table 1. Chemical formulae, structure, molecular weight and solubility of the Pharmaceuticals (Wishart et al., 2018).

| Name          | Chemical formula & structure | Mol. wt. (g/mol) | Therapeutic use     | Solubility in water at r.t.p (g/L) | Degradation (%) |
|---------------|------------------------------|------------------|---------------------|-----------------------------------|-----------------|
| Atenolol      | C14H22N2O3                  | 266.34           | Anti-hypertensive   | 26.5                              | 6.8 ± 0.026     |
| Ciprofloxacin | C17H18FN3O3                 | 331.35           | Broad spectrum antibiotic | insoluble                  | 0.0             |
| Isoniazid     | C6H7N3O                     | 137.34           | Anti-tuberculosis   | 140                               | 0.8 ± 0.003*    |
| Mebendazole   | C12H15N3O3                  | 295.29           | Anti-helminth       | 0.0713                            | 13 ± 0.050      |
| Nevirapine    | C15H14N4O                   | 266.89           | Anti-retroviral     | 7.046 × 10⁻⁶                      | 1.3 ± 0.005     |
| Penicillin-v  | C16H18N2O5S                 | 350.39           | Antibiotic          | 0.454                             | 1.0 ± 0.004     |
| Quinine sulfate | 2(C20H24N2O2).2H2O.H2SO4   | 782.94           | Antimalarial        | 0.334                             | 1.8 ± 0.008     |
| Rifampicin    | C43H58N4O12                 | 822.94           | Anti-tuberculosis   | 1.3                               | 0.8 ± 0.003*    |
| Ketoconazole  | C26H28Cl2N4O4               | 531.43           | Antifungal          | 0.0866                            | 1.0 ± 0.003     |

* Tested as a combination therapy of isoniazid/Rifampicin called Rifamate.
bioaccumulation. Of the eight pharmaceuticals, ciprofloxacin, phenoxymethylpenicillin (pen-V), quinine sulfate nevirapine and rifamate were tested for fourteen days after which the tests were ended because they had three consecutive plateau determinations and it was concluded that the degradation had stopped, all these pharmaceuticals were found to be non-biodegradable.

The other three pharmaceuticals, atenolol, ketoconazole, mebendazole were tested for a total of 28 days and were all found to be non-biodegradable as well.

The biodegradability of Ciprofloxacin was found to be 0.0%, which is consistent with results obtained by Kümmerrer et al., in 2000 (Kümmerrer et al., 2000) in which Ciprofloxacin biodegraded 0.0% in 28 days. Ciprofloxacin is a broad spectrum drug potent against both gram negative and gram positive bacteria. It is therefore fair to assume that there was inhibition to some extent of the bacteria by the test substance, which led to a negative biodegradation on the third, fifth and ninth day after correction of the blank values. The low EC50 values of ciprofloxacin against Daphnids and algae is also an indication of its high toxicity against the inoculum (Vankova, 2010; Sanderson and Thomsen, 2009).

Phenoxymethylpenicillin (Pen-V) biodegraded 1.0 ± 0.0044% during the test period, no literature data on the biodegradation percent of Pen-V was available at the time of writing this manuscript but studies have been done to determine the concentrations of Pen-V in the environment (Richardson, 2007) and also its removal from Waste Water Treatment facilities indicating that the drug is a persistent pollutant and is therefore non-biodegradable (Gulkowskaa et al., 2008).

The anti-tuberculosis drug recommended in Zambia is a combination of isoniazid and rifampicin known as Rifamate. This mixture biodegraded 0.8 ± 0.003% during the test period. Literature data on the biodegradation of the mixture is missing but Isoniazid was found to biodegrade 1.0% by Vankova in 2010 (Vankova, 2010). The low acute oral (AO) LD50 dose and low EC50 (Sanderson and Thomsen, 2009) values for Daphnids and for algae suggested that isoniazid was toxic to sludge bacteria which contributed to the low biodegradation levels.

Ketoconazole biodegraded 1 ± 0.003% during the test period. Results were considered valid because the experimental setup was tested and found functional by comparing with the control and compared with Vankova (2010).

Mebendazole degraded 13 ± 0.050% during the test period and was therefore declared non-biodegradable. The percent biodegradation was higher than the background data (Vankova, 2010) where it biodegraded 2%. The degradation started on the third day at 2.2% and proceeded to 13.0% on the twenty-sixth day while the twenty-eighth day gave a percent of 12.3%. The drop in percentage could not be explained using the criteria for the test method used but the overall period helped in classifying this drug as non-biodegradable.

Nevirapine biodegraded 1.3 ± 0.005% during the fourteen days’ test period, Similar biodegradation studies done on nevirapine were carried out in 2010 by Vankova in which it biodegraded 3.0%. Nevirapine has a long half-life, high toxicity to higher organisms (AO) in rat is 400 mg/kg and high stability when exposed to light which makes it a drug of high concern.

Quinine sulfate biodegraded 1.8 ± 0.008% during the fourteen-day test period, biodegradation started on the fifth day and remained constant for the last seven days. Results were considered valid because the experimental setup was tested and found functional by comparing with the control and compared with Vankova (2010) as outlined previously. The low EC50 values against bacteria suggest quinine sulfate is toxic to activated sludge.

Atenolol biodegraded 6.8 ± 0.026% during the twenty-eight days’ test period, the biodegradation started on the sixth day with 1.1% and reached 6.8% on day twenty-eight. Results were considered valid because similar results by Xu et al. (2017) when they used an enriched nitrifying sludge containing ammonia oxidizing bacteria.

All biodegradation results were compared with the recommended standard, dextrose monohydrate which degraded 77 ± 0.270%.

4. Conclusions

The biodegradability of eight drugs commonly prescribed at the University of Zambia clinic (sampled using the convenient method of sampling by simple questionnaire admission and analysis of results) was measured using a very cheap but efficient and reproducible carbon dioxide evolution method according to the OECD guidelines (OECD, 1992) of testing for readily biodegradable compounds. Analytical grade dextrose monohydrate (Heberer, 2002; Vankova, 2010) was used as a control in this research work. It biodegraded 77.0 ± 0.270% in seven days. This was used to obtain the ideal biodegradation curve and also test the functionality of the system. All the pharmaceuticals tested were non-biodegradable during the test period which lasted 28 days or less depending on the biodegradation trait of the pharmaceutical. Mebendazole biodegraded the most with a percent biodegradation of 13.0 ± 0.050% followed by atenolol which biodegraded 6.8 ± 0.026%, quinine sulfate biodegraded 1.8 ± 0.008%, nevirapine biodegraded 1.3 ± 0.005% while phenoxymethylpenicillin (Pen-V) biodegraded 1.0 ± 0.004%. Ketoconazole biodegraded 1.0 ± 0.003% and isoniazid/rifampicin and ciprofloxacin biodegraded 0.8 ± 0.003% and 0% respectively. The combination drug was treated as a whole and hence total carbon was contributed by both drugs. Overall the low solubility of the pharmaceutical products analysed in this work (Table 1), needs a careful look as this might be one compounding characteristic influencing low biodegradability.

The biodegradation of pen-v is presented for the first time under carbon dioxide evolution test methods.

5. Recommendations

The biodegradability of ciprofloxacin as well as rifamate needs to be done using suitable systems to correctly and accurately evaluate their degradation profiles under this method.
Declarations

Author contribution statement

James Nyirenda: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Alexina Mwanza: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Lengwe Chilufya: Performed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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