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**DEGRADATION OF CYTOTOXIC COMPOUNDS
BY TiO₂-UV PHOTOCATALYSIS**

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ABSTRACT

Cancer is one of the most common causes of death and an increase in cancer cases is expected in next decades. As a consequence, the consumption of cytotoxic drugs is raising. These pharmaceuticals are able to interact with DNA blocking the proliferation of cancer cells but lack of selectivity and may act on healthy cells too. Unfortunately, these compounds have been detected in surface waters. In fact, wastewater treatment plants are not able to remove cytotoxic compounds due to their low concentrations and high hydrophilicity. Thus, it is important to develop new techniques capable to treat them. Photocatalysis through UV and TiO_2 seems an effective alternative as it involves the generation of highly reactive radical species. With the present work photocatalysis with Degussa P25 TiO_2 is tested on three widely used anticancer drugs, methotrexate (MTX), cyclophosphamide (CP) and gemcitabine (GEM). The effect of the process parameters, such as pH, TiO_2 load and initial concentration was investigated for MTX in pure water. pH plays an important role as it regulates adsorption and electrostatic interactions between MTX and TiO_2 . At acidic pH photocatalysis was completely inhibited while at neutral and basic pH degradation of MTX was possible. The degradation rate increased with increasing TiO_2 load reaching a plateau for $[\text{TiO}_2]=1.5$ g/L and with decreasing MTX concentration. At the optimal conditions MTX was completely degraded in less than 10 minutes. Nevertheless, degradation did not occurred in synthetic urine proving that inorganic ions and NOM inhibit photocatalysis. Results on a mix of MTX, GEM and CP underlined that MTX was still eliminated as CP and GEM showed only partial degradation. This may be due to the different reciprocal affinity with radical species. So, photocatalysis seemed applicable for cytotoxic drugs but further studies are needed to assess its applicability on complex matrixes such as hospital wastewaters. In addition, a more detailed study of the toxicity of the treated water should be performed to investigate the production of toxic by-products.

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1 INTRODUCTION

The present study focused on the photocatalysis through TiO_2 and UV irradiation of three widely diffused anti-cancer pharmaceuticals methotrexate (MTX), gemcitabine (GEM) and cyclophosphamide (CP), paying more attention on MTX since few studies have been performed about it.

The influence of parameters such as pH, photocatalyst load, cytotoxic compound concentration and irradiation wavelength was studied to firstly optimize the process on MTX.

After assessing and optimizing photo-degradation of MTX the technology was tested on a mixture of MTX, GEM and CP since hospital wastewaters contain more than one contaminant with worse effects on the environment.

The present master thesis aims to understand what are the main parameters influencing photocatalytic degradation of cytotoxic compounds in order to support the *TREAT-AFTER-TOO project*; its final aim is to design a Photocatalytic Membrane Reactor (PMR) to test at a pilot scale in hospitals in India and Portugal (www.indigoprojects.eu/object/project/118). PMRs couple photocatalysis and membrane filtration allowing the set-up to work in continuous flow and recover TiO_2 particles through the filtration step. The work was performed in the context of an exchange program with the Ecole Polytechnique de Louvain (Louvain-la-Neuve, Belgium) where all the laboratory activities were carried out.

The project involves the use of a copper-doped TiO_2 since the doping permits the photocatalyst to be activated by low frequency light radiation offering the possibility to exploit solar irradiation. The recovery of TiO_2 particles and the use of sunlight configure this treatment as a sustainable technology that combines economic and environmental advantages.

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The fight of cancer is one of the most important medical challenge of the 21th century since nowadays it still represents one of the most common cause of death. Among all the illnesses, only cardiovascular disturbs kill yearly more people than cancer (Stewart et al., 2014).

According to 2014 World Cancer Report by WHO, in 2012 14.1 millions of new cases of cancer arose leading to the death of 8.2 millions of people. Since the 1950's cancer incidence have been increased (Figure 2.1) and it is expected to continue increasing with the growth in world population and the improvement in life conditions in developing countries that will characterize the next decades. The relationship between cancer incidence and developed countries lifestyle will bring to 20 million new cases of cancer by 2025 (Figure 2.2).

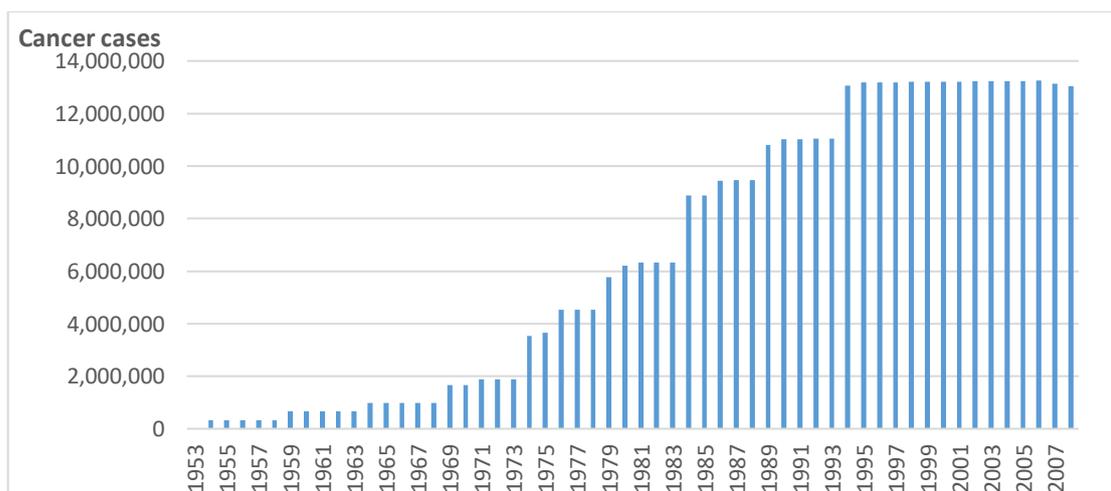


Figure 2.1: Incidence of cancer during time. Data from Global Cancer Observatory, IARC (<http://gco.iarc.fr/>)

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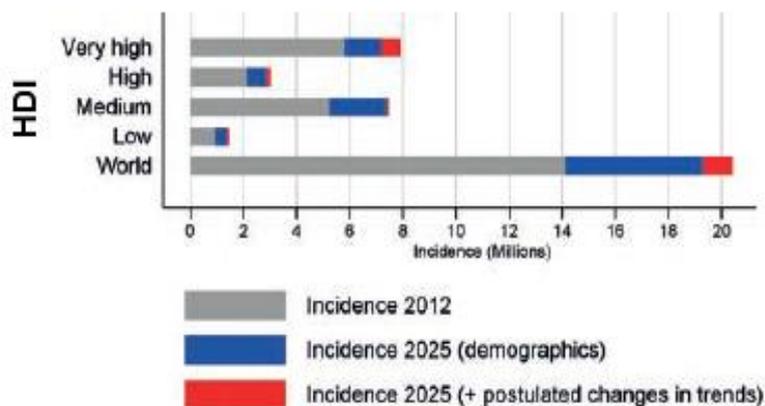


Figure 2.2: Relationship between cancer incidence and Human Development^(*) Index, Incidence in 2012 and estimated incidence in 2025 (Stewart et al., 2014).

In blue the change of incidence due to the growth of population, in red the change due to the postulated evolution of human habits

Along with the increase in cancer cases more pharmaceuticals will be consumed to cure new patients and this will constitute an alarming threat for the environment.

These compounds, known as cytotoxic or antineoplastic compounds, are designed to interact with DNA by altering its structure in order to rapidly inhibit the growth of cancer cells but they lack of selectivity and cause cytotoxicity to normal cells too.

Many of these pharmaceuticals proved to have genotoxic and mutagenic effects on aquatic microorganisms (Česen et al., 2016; Kümmerner et al., 2000; Zounková et al., 2007). Among all these pharmaceuticals, 5-fluoracil, ifosfamide, cyclophosphamide and methotrexate are the most administered anti-cancer worldwide (Kosjek & Heath, 2011).

However, many other pharmaceuticals belong to the group of the anticancer drugs and consumption trends vary regionally and yearly. For example in Italy, cyclophosphamide is one the most consumed drugs (with an average of 118±7 t/year in the period 2014-2016) as its average yearly consumption is higher than the one of other common chemotherapeutic agents. Taking into account methotrexate and gemcitabine, average cyclophosphamide yearly consumption is more than 6 times higher than the one of methotrexate and 270 times higher than gemcitabine consumption.

Nevertheless, if the consumption of cyclophosphamide and methotrexate was almost stable during the period 2014-2016, the consumption of gemcitabine increased by the

4 ^(*) The Human Development Index measures the development of a country taking into account the healthcare level (life expectancy at birth), the education level (expected and average years of scholarship) along with the economic level (per capita gross net income). (<http://hdr.undp.org/en/content/human-development-index-hdi>)

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187% (*elaborazione AIFA su dati NSIS - Flusso della Tracciabilità del farmaco, D.M. 15 luglio 2004 - Ministero della Salute*).

Thus, there is a need to monitor the highest possible number of these compounds to prevent environmental pollution.

Cytotoxic compounds are excreted by patients taking chemotherapy and enter the sewerage through hospital wastewaters (HWWs) or municipal wastewaters (MWWs) depending on the time spent at the hospital. Via treatment plants they can reach surface waters as conventional treatment plants are not able to degrade them due to their high persistency and low adsorption on activated sludge flocs.

Some studies arose the possibility that these contaminants could also reach drinking water at very low concentrations leading to possible harmful effects on human health (Franquet-Griell et al., 2016); no study about long time human exposure at very low concentrations has been conducted yet.

For all these reasons, anti-cancer drugs caught the attention of the scientific community that started to monitor them through international risk-assessment programmes as PHARMA (<http://pharmas-eu.net/>) and CytoTreat (<http://www.cytothreat.eu/>).

As monitoring can help us to understand dangers to ecosystems and human health, source control can avoid any risk on man and nature. As stated before, MWWs and HWWs are the principal sources of these pollutants, with the latter playing the most important role. It is thus necessary to equip hospitals with proper treatment units for their effluents.

Among all possible treatments, Advanced Oxidation Processes, AOPs, with the employ of very reactive radical species, showed promising results in cytotoxic compounds degradation. Processes using ozone, UV radiation, H₂O₂ and Fenton reactions proved to be possible alternative to HWWs treatment but nowadays scientific research focused on light-activated photocatalyst treatment using TiO₂.

Photocatalysis is a technique that combines effective degradation with economic and environmental benefits as TiO₂ has a quite low cost and its chemical stability and good mechanical properties make it recoverable combining photocatalysis with membrane filtration.

In addition, experiments on doped TiO₂ proved that doping, typically with noble metals, could be a possible way to activate the photocatalyst under visible light (Fiszka

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Borzyszkowska et al., 2016; Ofiarska et al., 2016) leaving open the possibility of exploiting sunlight as a free source of activation energy.

2.1 Antineoplastic compounds

Cytotoxic compounds belongs to the group L (Antineoplastic compounds) of Anatomical and Chemical Therapeutic Classification (ATC). Cytotoxic compounds can furtherly be divided in two classes: cytotoxic, strictly so-called, and cytotoxic compounds.

Cytotoxic compounds act altering the DNA of the cells leading to their death as cytostatic compounds do not modify DNA but blocks cellular reproduction targeting especially highly reproductive cells such as cancer cells.

In Table 2.1 it is reported a re-worked version of the synthetic classification by Besse et al. (*Besse et al., 2012*). The classification is not strict as some drugs show multiple mechanisms of action.

Due to their interaction with DNA and cellular reproduction these compounds are very dangerous as they can theoretically damage all eukaryotic organisms (*Johnson et al., 2008*). For this reason, they are considered as Contaminants of Emerging Concern (CECs) namely pollutants with known or suspected adverse effects on environment or human health whose release in water has not been regulated yet.

These chemicals proved to be toxic, genotoxic, carcinogenic and mutagenic to microorganisms (*Česen et al., 2016; Mater et al., 2014; Zounková et al., 2007, 2010*). The results of the common ecotoxicity tests have been reviewed by different authors (*Besse et al., 2012; Kosjek & Heath, 2011; Zhang et al., 2013*).

As these compounds are present as a mixture of different drugs, it is important to underline that a mix of cytotoxic compounds is more harmful than a single compound (*Elersek et al., 2016; Mater et al., 2014*).

However, usually ecotoxicity studies are carried out at higher concentration (µg-mg/L) with respect to environmental ones (low ng/L) and address mainly acute toxicity. To the best of author's knowledge, till now no study has been performed at environmental concentrations and the effect on long time exposure at trace levels is not known. For these reasons other authors underline the need of long time and even

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multi-generation toxicity tests at concentration that can be found in real waters (*Besse et al., 2012*).

Table 2.1: Classification of cytotoxic drugs (re-worked version of *Besse et al., 2012*).

Class	Mechanism of actions	Detailed mechanism
Cytotoxics that interacts directly with DNA	Alkylating agents <i>Cyclophosphamide, Ifofosfamide</i>	Attach one or more nucleophile groups to the DNA and inhibit or alter its transcription
	Platinum complexes <i>Cisplatin, Carboplatin</i>	Prevent DNA replication by binding to it
	Intercalating agents <i>Doxorubicin, Daunorubicin, Mitoxantrone</i>	Break single-stranded DNA
Cytotoxics that interacts indirectly with DNA	Antimetabolites <i>Methotrexate, 5-Fluoracil, Gemcitabine, Capecitabine</i>	Structural analogues of purine, pyrimidine or folic acid, act by blocking enzyme activity and DNA synthesis
	Cytotoxic antibiotics <i>Doxorubicin, Epirubicin, Mitomycin C</i>	Intercalate between DNA base pairs disturbing the synthesis and/or the functioning of nucleic acids
	Mitotic spindle inhibitors (Plant alkaloids) <i>Vinblastine, Vincristine</i>	Halt chromosome segregation by inhibiting mitotic spindle formation
	Topoisomerase inhibitors <i>Irinotecan, Etoposide</i>	Induce or stabilize DNA damage by blocking relegation of double stranded DNA
Cytostastics	Protein kinase inhibitors <i>Imatinib</i>	Interact with protein kinases, involved in the regulation of many biological processes (cell growth, migration, survival...)
	Monoclonal antibodies <i>Brentuximab vedotin, TDM-1</i>	Block tumoral cells extracellular receptors

Regarding human exposure, cytotoxic compounds prove to have noxious effects on hospital staff working with the drugs (*Bouraoui et al., 2011*) but the concentration of the administered drugs are much more higher than the one in the environment. Nevertheless, humans may be exposed to concentration still non detectable with actual analytical tools through drinking water during the whole life. The effects on

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long time exposure are not known yet and the teratogenic effect of antineoplastic compounds could be an issue for pregnant women (*Johnson et al., 2008*).

2.1.1 Environmental properties

Cytotoxic compounds are typically organic polar compounds possessing a broad range of different physico-chemical properties. Regarding the parameters that affect the fate and the distribution of these compounds in the environment, these includes:

- 1) solubility;
- 2) Henry's coefficient (K_H);
- 3) vapor pressure;
- 4) the dissociation constant pK_a ;
- 5) the octanol-water partition (K_{ow}) and distribution coefficient (D_{ow});
- 6) organic carbon partition coefficient (K_{oc});
- 7) the bioconcentration factor (BCF);
- 8) atmospheric OH \cdot rate.

The physico-chemical properties of some of the common used cytotoxic drugs are reported in Table 2.2.

Cytotoxic compounds have generally high values of *solubility* and low values of the *Henry coefficient* K_H and *vapor pressure* so removal by precipitation or volatilization is negligible.

The *dissociation constant* pK_a gives information about the state of dissociation of a compound in a solution at specific pH. In particular, if $pH > pK_a$ the compound tends to dissociate and it is more mobile in the aqueous phase.

Considering an environmental pH of 7, MTX, IF and CP are likely to be dissociated (Table 2.2).

pK_a influences the ionization state of the compounds since compounds tend to be deprotonated if $pH > pK_a$ or protonated otherwise. The state of protonation regulates electrostatic interactions with the typically negative charged organic matter and other solid in the water matrix promoting or preventing adsorption.

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The *octanol-water partition coefficient* K_{ow} , generally reported as $\log K_{ow}$, gives indication about the hydrophilicity or hydrophobicity of a compound. Namely, it is defined as the ratio between the concentration of the un-ionized form of a compound in octanol and its concentration in water if the compound is dissolved in a mixture of the two immiscible phases (Eq. 2.1).

$$\log K_{ow} = \log \left(\frac{[C]_{octanol}^{un-ionized}}{[C]_{water}^{un-ionized}} \right) \quad \text{Eq. 2.1}$$

where $[C]$ is the concentration of a general compound.

Octanol is taken as a reference solvent as it is apolar and it mimics organic carbon in soils and sediments or in lipid tissues. The values of K_{ow} give also an indication about the sorption on activated sludge.

Apart from CP and IF, the other cytotoxic compounds have negative values of $\log K_{ow}$ showing high hydrophilicity. Nevertheless, CP and IF proved not to be adsorbed onto activated sludge (Kümmerer *et al.*, 1997).

However, the logarithm of the *distribution coefficient* ($\log D_{ow}$) should be taken into account when a compound can dissociate in water. In fact, $\log D_{ow}$ refers to both the ionized (dissociated) and un-ionized (un-dissociated) forms of the compound (Eq. 2.2)

$$\log D_{ow} = \log \left(\frac{[C]_{octanol}^{un-ionized} + [C]_{octanol}^{ionized}}{[C]_{water}^{un-ionized} + [C]_{water}^{ionized}} \right) \quad \text{Eq. 2.2}$$

It is possible to calculate the value of D_{ow} from K_{ow} through Eq. 2.3.

$$D_{ow} = K_{ow} + 1 / (1 + 10^{|pH-pKa|}) \quad \text{Eq. 2.3}$$

The *organic carbon partition coefficient* K_{oc} is similar to K_{ow} as it expresses the ratio between the concentration of the contaminant adsorbed to organic carbon in soil and the concentration of the compound in solution in the water phase.

Compounds with high values of this coefficient are less mobile in soil and accumulate. As expected by low values of $\log K_{ow}$ cytotoxic compounds have relatively low values of K_{oc} due to their strong hydrophilicity.

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The *bioconcentration factor* BCF is the ratio between the concentration of a chemical in an aquatic organism and the concentration in water. Usually, the partition coefficient K_{ow} and the BCF are linked as the affinity for lipid translates into bioaccumulation in organisms. So, it is expected that a chemical with a low value of K_{ow} has a low BCF.

According to Franke the bio-magnification risk can be extrapolated from BCF data: the risk is low if $BCF < 30$, moderate if it is between 30 and 100, high if between 100 and 1000 and very high if higher than 1000 (*Franke et al., 1994*).

Data from Table 2.2. show that the risk of biomagnification related to anticancer drugs is low.

In the end, *atmospheric OH· rate* describes the reaction rate of a compound with hydroxyl radicals and it is a rough indicator to assess the potential of a pollutant to be degraded by AOPs. Among the cytotoxic compounds, MTX seems to be more easily degraded by hydroxyl radicals. In fact, in a study with Degussa P25 TiO₂ and UV irradiation Lutterbeck reported an half-life of MTX of 3 min (*Lutterbeck et al., 2015*) as a similar study with IF and 5-FU reported respectively half-lives of 10,9 and 20,9 min (*Lin & Lin, 2014*). The half-lives of the three compounds are in accordance with value of the atmospheric OH· rate in Table 2.2

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Table 2.2: Physico-chemical properties of common used cytotoxic compounds.

(MTX=methotrexate, CP=cyclophosphamide, GEM=gemcitabine, 5-FU=5-fluoracil, IF=ifofosfamide)

	pK _a	Solubility (mg/mL)	logK _{ow}	K _{oc}	BCF	Atmospheric OH ⁻ rate (10 ⁻¹¹ cm ³ /molecule×s)	Henry law constant (atm·m ³ /mole)	Vapor pressure (mm Hg)	UV max
MTX	4,7 [1] 4,8-5,5 [2]	0,45 (pH=5) 9,97 (pH=7) [3] 2,6 [1]	-1,85 [1]	1 [1] 20 [4]	3,2 [1] 3 [4]	31,7 [1]	1,54×10 ⁻³¹ [1]	2,09×10 ⁻¹⁹ [1]	290 [5]
CP	2,84 [5] 6 [4] 4,5-6,5 [6]	40 [1]	0,63 [1]	52 [1] 59 [5]	3 [1] 2,1 [5]	7,03 [1]	1,40×10 ⁻¹¹ [1] 7×10 ⁻¹¹ [5]	4,45×10 ⁻⁵ [1]	200 [5]
GEM	3,6 [1] 5,27 [5] 11,65 [8]	51,4 [1] 15,3 [4]	-1,22 [1] -1,24 [4] -2,01 [5] -2,22 [9]	1 [4] 1,4 [5]	3 [1] 1 [5]	0,04 [5]	1,7×10 ⁻¹⁷ [1]	1,7×10 ⁻⁹ [7]	234; 268 [5]
5-FU	8; 13 [1] 8,02 [5] 7,6-8; 13 [4]	11,1 [1]	-1 [1] -0,89 [5] -0,93 [4]	8 [1] 4 [4]	3,6 [1] 3 [5]	0,583 [1]	1,66×10 ⁻¹⁰ [1]	2,68×10 ⁻⁶ [1]	266 [5]
IF	1,45 [5] 1,45-4 [4]	3,78 [1]	0,86 [1]	70 [1] 62 [5] 51 [4]	3 [1] 2,2 [5]	4,28 [1]	1,36×10 ⁻¹¹ [1]	2,98×10 ⁻⁵ [1]	<290 [5] 200 [10]

[1] Kosjek & Heath, 2011 [2] Reid et al., 1993 [3] Rubino, 2001 [4] Booker et al., 2014 [5] Zhang et al., 2013 [6] L. Wang et al., 2009 [7] Toolaram et al., 2014 [8] Santana-Viera et al., 2016 [9] Negreira et al., 2013 [10] Fiszka Borzyszkowska et al., 2016

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Two other important environmental properties not related to specific indexes are biodegradability and the stability towards photolysis.

The *biodegradability* of a compound deeply influences its fate in a wastewater treatment plant and in the environment. Typically cytotoxic compounds are persistent and recalcitrant pollutants difficult to degrade by biological treatments but biodegradability depends also on the chemical composition and structure of the compounds and cytotoxic drugs gather many different chemical compounds.

IF and CP proved not to be biodegraded both in laboratory tests and by WWTPs (Kosjek & Heath, 2011). 5-FU and MTX have been degraded in laboratory at conditions similar to that of a real WWTP but degradation of MTX led to the formation of a recalcitrant biodegradation product, 7-hydroximethotrexate (Kiffmeyer et al., 1998). Also GEM was found to be biodegradable in laboratory tests (Kümmerer et al., 1997).

Another important property that influences the stability of anticancer drugs in the environment is capacity to absorb solar light. An organic compound is able to absorb light if it contains *chromophores*, namely functional groups that permits light absorption. In particular, chromophores have to allow light absorption in the range of UV-vis wavelengths (200-800 nm) that represent the highest fraction of the solar irradiation spectrum. Typically chromophores containing aromatic rings and double bonds, for example, C=C, C≡C, C=O and N=O, are able to absorb lower wavelengths in the range 200-800 nm. Other important chromophores are functional groups containing hetero atoms (i.e. atoms of elements different from C or H) with non-bonding electron pairs like C-Br. These other functional groups absorb light at higher wavelengths and are characterized by lower molecular extinction coefficients (so they are more likely to absorb light in their absorption spectrum).

The absorption of UV-vis light is promoted by the presence of conjugated bonds and as a general rule the more are the conjugated bonds the more the molecule is able to absorb light at lower energy (higher wavelength) (Decadic, Extinction, & Pollutants, 2003).

Among the compounds in Table 2.2, MTX with is suitable for photolysis due to the three aromatic rings and the presence of C-O double covalent bonds (Figure 2.3).

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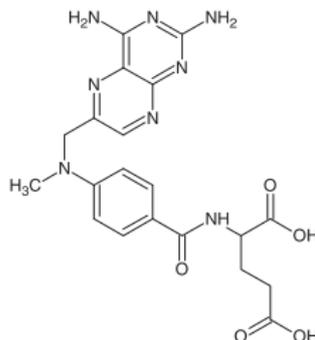


Figure 2.3: Methotrexate (MTX) structure

Nevertheless, other drugs do not absorb light in the UV-vis range. 5-FU proved not to absorb light at wavelengths higher than 290 nm as CP and IF react only with short wavelengths (<190 nm) (Baumann & Preiss, 2001).

In summary, cytotoxic compounds represent a class of high persistent compounds in water as:

- they are very soluble hydrophilic compounds (high solubility, very low K_H);
- they do not sorb to soil or sediments so they are likely to be present in surface water (high values of K_{OW} and K_{OC});
- they are generally low biodegradable and do not adsorb on sludge making the common activated sludge treatment unsuitable for their removal.

However, their interactions with hydroxyl radicals suggest that AOPs may be a possible way to degrade them.

2.2 Sources of pollution

The two principal pathways through which cytotoxic compounds can contaminate the environment are hospital and municipal wastewaters. Patients taking chemotherapy excrete a fraction of the ingested drugs through urine and faeces in the sewerage. Drugs are not completely metabolized by human body and are excreted unchanged or in the form of metabolites. The excretion percentage depends on the type of drug and its way of administration (oral or intra-venous), the age and the constitution of the patients and the time of administration (K. Kümmerer et al., 2000; Pérez & Barceló, 2007).

These factors influence also the form of the excreted drugs in the urine. In fact, drugs that need to be activated by enzymes (*pro-drugs*) are mainly excreted in the form of

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metabolites that should be taken into account when investigating the occurrence of cytotoxic compounds in the environment. At the European level (Directive 2004/27/EC), environmental risk assessment is required for pharmaceuticals human metabolites with excretion ratios exceeding the 10% of the total dose (*Laenge et al., 2006*).

As in the past HWWs were the main sources of pollution, currently the contribution of MWWs to environmental pollution is raising. In fact, an increasing percentage of this kind of pharmaceuticals is now administered to out-patients and it is sold in pharmacies. Nowadays, the 75% of cytotoxic consumption is at home (*Johnson et al., 2008*). In addition, the time spent at the hospital for chemotherapy is reducing and even if the patients take the drugs at the hospital, the excretion is at home.

Other minor sources of pollution derive from concentrated effluents of pharmaceutical plants and contamination from improper waste disposal (*Reddersen et al., 2002*).

Nevertheless, even if the concentration of these compounds in MWWs can be higher than in HWWs (*Ferrando-Climent et al., 2014*), typically they are more concentrated in hospital effluents. Weissbrodt et al. found that 2',2'-difluorodeoxyurine, the human metabolite of gemcitabine, reached a concentration of 200 mg/L in the wastewater of a hospital in Swiss (*Weissbrodt et al., 2009*).

Many countries still discharge hospital effluents into the sewer network without any treatment. For instance, in Europe there are no specific legislation or guidelines regarding the management of HWWs. The danger of hospital waste is recognized by European Directive n.98 of 19 November 2008 about the management of hazardous wastes that states that liquid wastes, such as pharmaceutical products, medicines and others (soaps, solvents etc), must be disposed as waste and not discharged into the sewer. However, there is no specific indication regarding hospital effluents which may contain these same dangerous substances (*Carraro et al., 2016*).

Another European directive, the European Directive n. 91 of 21 May 1991 (91/271/CEE modified from Directive 27 of February 1998 n. 98/15/CE) on the treatment of MWW requires a pre-authorisation for the discharge into MWW collection system of industrial effluents (*Carraro et al., 2016*). However, the decision of considering HWWs as industrial effluents or municipal ones is up to each EU state member. As an example, in Italy no pre-treatment is required for HWWs.

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Other countries such as Spain or France consider HWWs as industrial effluents for which specific characteristics are required. Nevertheless, it is possible that HWWs do not comply with these characteristics and are consequently treated as domestic wastewaters. In fact, in term of macropollutants (NOM and nutrients) and physico-chemical characteristics (pH, temperature, alkalinity...) HWWs are similar to MWWs (*Carraro et al., 2016; Verlicchi et al., 2015*).

Thus, as rarely hospitals have a water treatment unit, HWWs are discharged untreated in the sewerage where they mix and dilute with MWWs and reach a wastewater treatment plant (WWTP). The concentration of cytotoxic compounds released in HWWs depends on the type of hospital (specialized in cancer treatment or not), its size (number of beds), its water consumption (dilution) and on the presence of *in-situ* treatment units (removal).

WWTPs are not able to efficiently remove these pollutants as they are designed to remove principally nutrients and macropollutants at concentrations of mg/L. Inefficacy of WWTPs in cytotoxic drugs removal is documented by many authors (among them: *Buerge et al., 2006; Kümmerer et al., 1997; Verlicchi et al., 2010; Yin et al., 2010*).

For these reasons, some countries are raising awareness about the environmental danger of micropollutants; for example, in Switzerland a new treatment step for micropollutants (ozonation or adsorption on activated carbon) is mandatory for WWTPs starting from 2016 (*Wittmer et al., 2015*).

So, effluents of WWTPs typically still containing cytotoxic compounds at low concentrations (in the order of ng/L) are discharged in the environment leading to possible surface water (*Ferrando-Climent et al., 2014*), ground water (*Heberer, 2002*) or land contamination if treated waters are re-used for irrigation.

Cytotoxic compounds diluted in surface waters undergo a series of processes: they can partially biodegraded by microorganisms, they can be photodegraded by solar irradiation or they can adsorb to organic matter or accumulate in the sediments.

Regarding photodegradation, most antineoplastic compounds proved not to be directly photodegradable in natural environment. Degradation by radicals formed due to solar irradiation may be possible but only in clear water rich in nitrate ions since hydroxyl radicals form after photolysis of them (*Buerge et al., 2006*).

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Hence, as poorly degradable in the environment, antineoplastic compounds reach drinking water treatment plants (DWTPs) that treat surface water before distribute it to users. DWTPs involve advanced treatment steps as ozonation, chlorination and UV treatment that can act on these recalcitrant pollutants. However, some authors arose the possibility of drinking water contamination by the cytotoxic compounds itself or in the form of by-products (*Franquet-Griell et al., 2016; Garcia-Ac et al., 2010*). Actually, studies about the presence of these compounds in tap water revealed concentrations under the limit of detection of the used instruments (*Mendoza et al., 2016; Mompelat, Thomas, & Le Bot, 2009, 2011*) but more detailed studies including human metabolites and possible transformation products have not been conducted yet. The fate of cytotoxic compounds in the water treatment cycle is summarized by Figure 2.4.

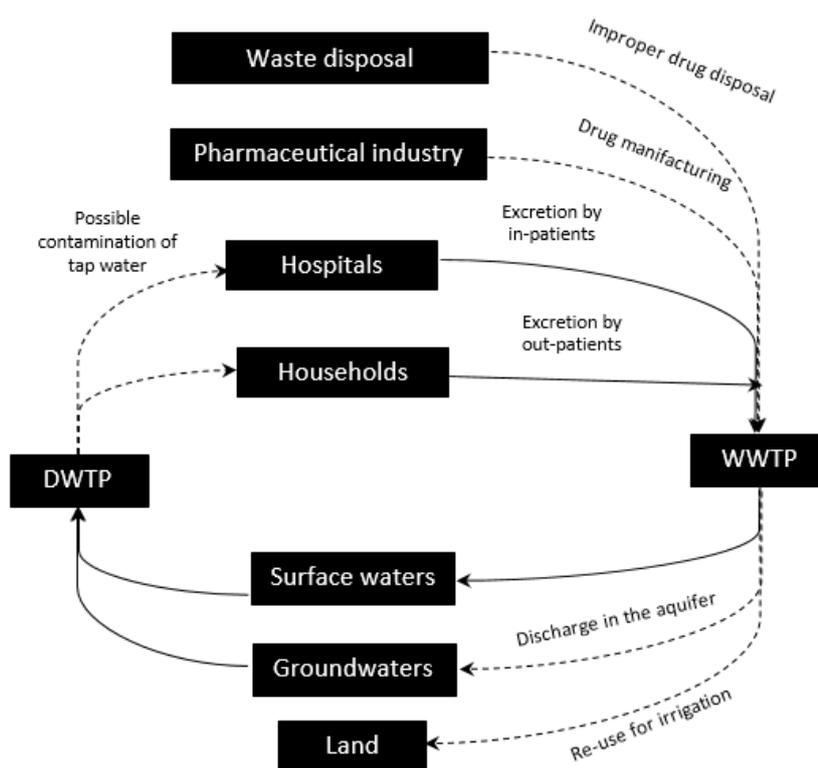


Figure 2.4: Transport routes of cytotoxic compounds in the environment.
Dash lines indicates uncertain routes

2.3 Treatment technologies

A more specific review by Zhang focused on the treatment of cytotoxic compounds (Zhang *et al.*, 2013) but almost all of the studied technologies were the same used for HWWs treatment in general. These technologies may be subdivided in three main groups:

- 1) *Advanced biological treatments*: membrane bioreactors (MBR);
- 2) *Physico-chemical separation*: nanofiltration (NF), reverse osmosis (RO), powdered activate carbon (PAC) and granular activated carbon (GAC);
- 3) *Advanced oxidation processes*: ozonation (O₃, O₃/H₂O₂, O₃/UV, O₃/H₂O₂/UV), oxidation with H₂O₂ (H₂O₂, UV/ H₂O₂), electrochemical oxidation, photocatalytic oxidation (UV-TiO₂), Fenton and photo-Fenton processes.

These technologies can be applied alone or in combination to enhance degradation of the compounds.

Before describing the functioning of each treatment option it is useful to distinguish between the different effects that each technique has on the contaminants.

First of all, a great distinction should be done when comparing processes that degrade or separate compounds from water.

Among the three categories listed above, advanced biotic treatments and AOPs actually degrade compounds in water as physical treatment processes only transfer the compounds from a phase to another (in the case of adsorption on activated carbon) or concentrate the compounds in another stream (the retentate in the case of NF/RO). As degradation sometimes may lead to by-products whose effects could be of concern in term of ecotoxicity, separation overcomes this issue. On the other hand, separation implies the treatment or disposal of the residues of the process with an increase in costs.

The difference between degradation and demineralization concerns the final products of the applied treatment. As degradation means the transformation of a parent compound into other low molecular weight species, demineralization is the conversion of an organic compound into CO₂ and elementary substances. Degradation is considered acceptable only to the extent that the toxicity of its products is lower

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than the one of the parent compound (*Eddy & AECOM, 2014*). In fact, a partial degradation may be acceptable due to the higher costs of a complete demineralization. On the contrary, a degradation is not acceptable if generates more toxic or less degradable species. Thus, demineralization yield should be adopted to compare different treatments as the disappearance of the parent compound does not necessarily result in better water quality (*Prasse et al., 2015*).

The formation of transformation products (TPs) concerns both advanced biological treatments and AOPs but generally the latter are more studied from this point of view due to their complicate degradation pathways. The degradation mechanism depends on applied AOP with different AOPs leading to same or different TPs. However, generally an increase in toxicity is observed during the first phase of an AOP treatment suggesting that high toxicity is associated to transient TPs and enough reaction time is necessary to avoid worsening the quality of the treated water (see Table 2.3).

Table 2.3: Review about TPs of common used cytotoxic compounds

Cyt	Treatment	TPs	Ecotoxicity	Ref.
MTX	UV/H ₂ O ₂	6 TPs	-	[1]
	UV/TiO ₂	6 TPs	-	
	UV/TiO ₂	8 TPs	Acute toxicity test: high toxicity of transient TPs, slight toxicity of final TPs	[2]
5-FU	UV/H ₂ O ₂	6 TPs	Chronic toxicity, genotoxicity and mutagenicity tests: no toxicity after 256 min of irradiation	[3]
	UV/TiO ₂	4 TPs	Genotoxicity and mutagenicity tests: no toxicity after 256 min of irradiation	
	UV/Fe/H ₂ O ₂	2 TPs	-	
CP	O ₃ , O ₃ /H ₂ O ₂	1 TP	-	[4]
	UV/TiO ₂	7 TPs	Acute toxicity: toxicity increased during the first phase of the treatment and the decreased. No toxicity after 6 h of irradiation.	[5]
IF	UV/TiO ₂	10 TPs	Acute toxicity: toxicity increased during the first phase of the treatment and the decreased. No toxicity after 6 h of irradiation.	[5]

[1] Lutterbeck et al., 2015; [2] Calza et al., 2014; [3] Lutterbeck, Wilde, et al., 2015; [4] Fernández et al., 2010

2.3.1 Advanced biological treatments: MBRs

MBRs combine biological degradation and microfiltration in one unit thanks to microfiltration membranes with nominal pore sizes ranging from 0.1 to 0.4 µm. They may be immersed in the reactor or placed in a separated module placed outside. In the first configuration the vacuum applied to the membrane allows water to be drawn through the membrane while solids are kept inside the reactor.

In the case of separated systems, the influent is pressurized and pumped through the membrane placed outside that allows the passage of purified water and retains solid biomass that is recirculated to the bioreactor.

In these systems cytotoxic agents are removed from the effluent due to adsorption on biomass and/or biological degradation.

Nevertheless, the cytotoxicity of these compounds may slow down bacterial activity and lowering the efficiency of the process it is (*Delgado et al., 2009*).

A pilot-scale MBR was tested by Mahnik et al. (*Mahnik et al., 2007*) to treat the effluent from an oncological ward and the concentration of 5-fluoracil and anthracyclines, a class of cytotoxic antibiotics, was monitored. 5-fluoracil was rapidly degraded as anthracyclines were removed (removal efficiency >90%) due to adsorption onto the biomass.

In another study, the technology was tested on HWW for the removal of CP, a poorly degradable compound. CP exhibits low degradation (<20%) for SRT up to 50 days (*Verliefde et al., 2007*). However, a similar study on MTX in domestic wastewater with inoculated activated sludge showed removals up to 80% but the measure of ecotoxicity of the permeate revealed residual ecotoxicity probably due to degradation bio-products (*Delgado et al., 2009*).

2.3.2 Physico-chemical separation

Among the feasible treatments for cytotoxic compounds membrane filtration (NF and RO) showed interesting results as adsorption on activated carbon seemed not to be effective alone due to low logK_{ow} of the antineoplastic compounds.

Stripping is not a feasible technique due to the low values of the Henry coefficient of these pharmaceuticals (Table 2.2).

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Membrane filtration: NF and RO

Membranes can reject compounds namely through three mechanisms: *size exclusion* or *steric hindrance effect*, *electrostatic interactions* and *adsorption on the membrane surface*.

The *steric hindrance effect* depends directly on the dimensions of the pores of the membrane and on the size of the molecules to be removed. In the case of cytotoxic and in general all the pharmaceuticals only nanofiltration and reverse osmosis proved to be effective in water treatment because of the relatively low size and molecular weight of these compounds (Verlicchi *et al.*, 2015).

Electrostatic interactions establish between the typically negatively charged polymeric NF/RO membranes and positively or negatively charged compounds in solution. Negative charge of the membrane is due to the deprotonation of the carboxylic acid groups at its surface. These mechanisms are strongly dependent on pH as both the charge of the membrane and state of ionization of the compounds in solution are related to pH. The charge of the membrane surface at a specific pH can be known from its point of zero charge as the protonation/deprotonation state of a compound can be derived by its pKa. So, considering the membrane to have a negative charge at pH of HWWs, negatively charged compounds are rejected by the membrane as neutral and positive compounds may be separated only due steric hindrance effects or adsorption.

Adsorption of compounds on the membrane is typical of hydrophobic compounds as NF membrane are highly hydrophobic. Hydrophobic substances adsorb onto the membrane and may be removed from the effluent but in the case of molecules lighter than the molecular weight cut-off of the membrane adsorption is only the preliminary step before their diffusion in the membrane to the permeate side.

In the case of cytotoxic compounds the first two mechanisms are predominant as adsorption may be excluded due to their high hydrophilicity.

Application on CP proved that removal by NF was high (<90%) only when the membrane operated at a low water recovery percentage (10%) (Verliefde *et al.*, 2007). Wang and others compared the performances of NF and RO membranes demonstrating that the rejection of CP was higher with RO membrane (Wang *et al.*, 2009).

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Adsorption on activated carbon

Adsorption on the activated carbon in the form of a powder in slurry phase (PAC) or packed in beds (GAC) has been tested to treat HWWs containing also cytotoxic drugs.

In the review by Verlicchi (*Verlicchi et al., 2015*) it is underlined that it was possible to remove CP and IF with PAC only at high dosage (150-450 mg/L). Capecitabine, the pro-drug of 5-fluoracil, showed low removal efficiency even if at high dosages (<40%). Due to the high concentration needed PAC seems not to be an economically sustainable technique.

However, GAC could be a more effective technique. In fact, Lenz et al. coupled an MBR with a GAC adsorption column to treat the effluent from the oncological ward of the hospital of Vienna managing to remove cancerostatic platinum compounds (CPCs) and 5-fluoracil with high removal efficiencies. As 5-fluoracil was already removed below the detection limit after the MBR step, the GAC column allowed the removal of CPCs (*Lenz et al., 2007*).

2.3.3 Advanced oxidation processes

AOPs involves the production of radicals to oxidise recalcitrant compounds. Among them, ozonation coupled with H₂O₂ and/or UV irradiation is the more consolidated technique but nowadays there is an emerging interest on other treatments such as photocatalytic oxidation with titanium dioxide, Fenton/photo-Fenton, electrochemical oxidation and sonolysis.

Another issue associated with ozonation and AOPs in general is the formation of by-products whose toxicity as to be monitored since they can be more dangerous than their parent compound.

Oxidation with O₃, O₃-H₂O₂, O₃-UV and UV-H₂O₂

Ozonation is a consolidated technique for the treatment of micropollutants in water. Ozone can directly attack organic contaminants or decompose to form ·OH· radicals due to its unstable nature in water.

Compared to hydroxyl radicals that can react with a broad range of functional groups ozone reacts more selectively attacking electron-rich moieties of organic substances

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such as functional groups containing unsaturated double or triple bonds. More in detail, compounds as (*Lee & von Gunten, 2010*):

- olefin, phenol, aniline, thiophenol, thiol and tertiary amine exhibit high reactivity with ozone,
- secondary amines, thioester and anisol an intermediate reactivity,
- primary amines and nitro group a slow reactivity,
- amides are no reactive.

An enhancement of the process is expected at alkaline pH. pH can be increased artificially or in alternative it is possible to add H₂O₂ to increase the production of hydroxyl radicals.

In fact usually ozone is not dosed alone but process combining ozone and H₂O₂, ozone and UV irradiation or ozone with both UV and H₂O₂ are applied for wastewater treatment.

NOM concentration is an important parameter influencing the process since it acts as a radical scavenger both for ozone and hydroxyl radicals.

In the case of ozone treatment, the formation of bromides is possible for matrix containing bromine compounds.

Applications of ozonation for HWW treatment have been documented by Verlicchi et al. (*Verlicchi et al., 2015*) who reported that oxidation of CP, IF and capecitabine was possible at relatively high ozone dosages (removal efficiency >80% for dosages of 2gO₃/DOC or 4.1-7.8 gO₃/TOC).

Garcia-Ac et al. (*Garcia-Ac et al., 2010*) studied the ozonation of MTX and CP in drinking water showing that MTX was rapidly oxidised by ozone as CP was less reactive. This can be explained by the chemical structure of MTX that contains amino groups, an aromatic ring and two N-containing aromatic groups (Figure 2.3) that are known to be moieties selectively attacked by ozone. CP was more recalcitrant and only reacted directly with ozone at CT (O₃ concentration × contact time) = 45 mg min/L which is a relatively high value compared to common values used in treatment plants. The possibility to degrade MTX, together with 5-fluoracil, cytarabine and azathioprine, by ozone was proved by other authors (*Pérez Rey et al., 1999*).

Ferre-Aracil et al. applied ozonation to a real HWW and found that among the cytostatic compounds in solution, namely ifosfamide, cyclophosphamide, irinotecan and capecitabine, cyclophosphamide was the hardest compound to degrade. An ozone concentration of 43.9 g/m³ and a reaction time of 30 min were needed to remove the

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97% of CP in solution. The same authors estimated the cost of the treatment (cost of electricity + cost of oxygen supply) to be around 0.3 € per m³ of treated water (*Ferre-Aracil et al., 2016*).

The degradation of CP by ozonation was studied also by Lester et al. who underlined that the presence of NOM and alkalinity in treated water results in a slowdown of the reaction with the major effect of alkalinity (*Lester et al., 2013*).

In another study the same authors compared the performances of ozonation alone with treatments combining ozone and UV and/or H₂O₂. UV/H₂O₂/O₃ treatment led to highest degradation rates compared to the other treatments (*Lester et al., 2011*).

It is possible to generate hydroxyl radicals combining UV and H₂O₂ without O₃. This alternative may be feasible for micropollutants rather than high concentration contaminants as H₂O₂ has a small molar extinction coefficient.

In alternative, H₂O₂ can be added during ozonation to enhance the production of hydroxyl radicals or can be dosed in combination with UV.

Thus, organic compounds are degraded through two mechanisms: direct photolysis by UV and reactions with radicals.

Regarding applications on cytotoxic compounds, studies exist only at the laboratory scale.

Lutterbeck et al. compared the efficiency of three different UV-based treatments (UV/Fe/H₂O₂, UV/H₂O₂ and UV/TiO₂) for the treatment of MTX and 5-FU (*Lutterbeck et al., 2015a; 2015b*).

Regarding MTX, best demineralization efficiency was obtained with UV/Fe/H₂O₂ (78.4%) followed by UV/TiO₂ (72.1%) and UV/H₂O₂ (65.1%); the fastest process was UV/TiO₂ since it completely eliminated the parent compound in 4 min as with UV/Fe/H₂O₂ and UV/H₂O₂ the total elimination occurred after 8 min and 16 min respectively.

The study about 5-FU underlined no substantial differences in the demineralization achieved with the three AOPs and assessed that UV/Fe/H₂O₂ was the fastest process followed by UV/H₂O₂ and UV/TiO₂.

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Ecotoxicological analyses revealed that all the three AOPs reduced the ecotoxicity of the treated water in the case of 5-FU but transformation products of MTX after UV/H₂O₂ were still ecotoxic and less biodegradable.

Thus, results showed that H₂O₂ was more effective in combination with Fe (photofenton process) but further studies should be performed about the degradation pathways of other cytotoxic compounds as HWWs contain many different compounds whose TPs can be even dangerous than the parent compound.

In another study other authors tested different AOPs combining ozonation, UV irradiation and H₂O₂ to degrade CP. O₃/H₂O₂ was the fastest treatment followed by simple ozonation (*Lester et al., 2011*).

Cyclophosphamide can be degraded with UV/H₂O₂ too (degradation yield > 90%) but at high UV dose (1695 mJ cm²) (*Kim et al., 2009*).

Sonolysis

Sonolysis is the application of ultrasonic irradiation to a water medium to degrade organic compounds in solution. Ultrasounds cause acoustic cavitation that is the adiabatic formation and collapse of bubbles with the creation of microscopic zones at high temperature and pressure where a high amount of energy is liberated.

In these microzones temperature can reach 10,000 K and pressure is in the range 300-975 bar (*Crittenden et al., 2012*).

Sonolysis involves mainly two degradation mechanisms: pyrolysis of the organic pollutants due to high temperatures and pressures (*Weavers & Hoffmann, 1999*) or indirect degradation by radicals generated during the process.

In fact, water molecules affected by sonolysis evaporates leading to the formation of radicals and hydrogen peroxide in the bubbles (*Hartmann et al., 2008*).

Radicals may attack organic compounds on the gas-liquid interface at the surface of the bubbles or diffuse in the water medium (*Weavers & Hoffmann, 1999*).

Sonolysis can be coupled with ozonation to improve the production of hydroxyl radicals. The high energy liberated by cavitation allows the thermolytic decomposition of ozone and the subsequent formation of hydroxyl radicals (*Weavers et al., 2000*).

Somensì et al. (*Somensì et al., 2012*) combined ozonation with sonolysis to degrade MTX and doxorubicin (DOXO).

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DOXO resulted more difficult to remove (degradation efficiency <50%) as good results were achieved for MTX even without ozonation (degradation efficiency >80%). Coupling sonolysis with ozonation enhanced the performances of the process both for DOXO and MTX but the removal rate and efficiency was pH-dependent; at pH=7, that is close to the pH of HWW, the process achieved satisfactory results only for MTX (Somensi *et al.*, 2012).

Photocatalysis with UV-TiO₂

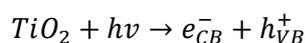
Heterogeneous photocatalysis is one of the most studied AOP for the removal of organic species in water treatment. During this process the photocatalyst is excited by light irradiation to directly oxidise organic pollutants or to favour the production of radical species that indirectly degrade the contaminants.

Many semiconductor metal oxide, such as ZnO, Fe₂O₃, CdS, GaP and ZnS, may act as photocatalysts but typically TiO₂ is employed due to its good thermal and chemical stability, its strong mechanical properties, its low excitation energy and its low cost. TiO₂ has three different crystalline forms anatase, rutile and brookite but usually commercial titanium-based photocatalyst are mainly composed by anatase and rutile. Degussa P25 TiO₂, the most employed photocatalyst for water treatment, is a mixture of 70-80% anatase and 20-30% rutile (Ohtani, 2008). The crystalline composition of the photocatalyst influences the band-gap energy, i.e the energy necessary to promote an electron from the valence band (VB) to the conduction band (CB) of the semiconductor. In fact, as anatase and rutile present band-gaps respectively of 3.2 eV and 3.0 eV the semiconductor needs at least 3.2 eV to be excited. This amount of energy can be provided by irradiation wavelengths shorter than 390 nm (Grčić & Li Puma, 2013).

Regarding the photocatalytic mechanism initiated by light irradiation, a brief description is provided by Colmenares and Xu (Colmenares and Xu, 2016) and by the reviews by other authors (Chong *et al.*, 2010; Herrmann, 1999; Ohtani, 2010, 2008; Fujishima, 2000, 2008).

When the photocatalyst surface is irradiated an electron moves from the VB to CB generating an electron-hole pair (Reaction 2.1).

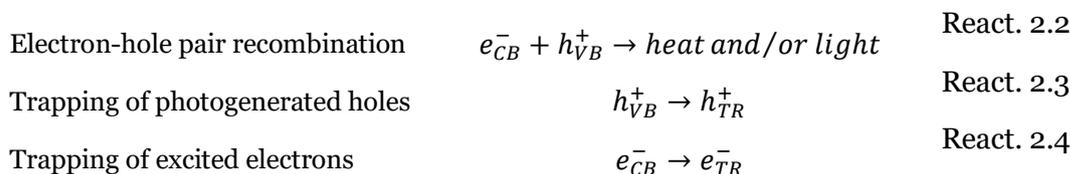
Electron-hole pair formation



React. 2.1

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The electron-hole pairs may recombine (Reaction 2.13) or if trapped at the TiO₂ surface (Reaction 2.2 and 2.3) they can lead to the production of radical species which can subsequently attack organic pollutants.



Trapped photogenerated holes and excited electrons react respectively with adsorbed water molecules and molecular oxygen to form hydroxyl radicals (Reaction 2.5) and superoxide anions (Reaction 2.6). These highly reactive and scarcely selective radicals are responsible in organic contaminants degradation.



The presence of electron scavengers such as adsorbed oxygen (Reaction 2.6) is vital to prolonge the life of electron-hole pairs as they typically recombines in nanoseconds. Another possible mechanism is the direct oxidation of organic contaminants by the trapped holes. In this case contaminants have to diffuse near the TiO₂ and to adsorb on an active sites where they are oxidised.

Typically, TiO₂ is dosed as powder in a slurry form but supported TiO₂ applications exist. TiO₂ may be supported on nanorods, nanofibers or membranes (*Chong et al., 2010; Colmenares and Xu, 2016*).

Nowadays, an interesting field of research is TiO₂ doping. The doping of TiO₂ with metal or non-metal impurities reduces the band-gap between the VB and CB allowing excitation of the photocatalyst with longer wavelengths. In fact, generally the photocatalyst is activated by UV irradiation provided by UV low pressure (254 nm) lamps that exert a synergic disinfection action or UV medium pressure lamps that provide a broader spectra in the range of wavelengths that can be absorbed by organic compounds (200-400 nm). So, the cost of the treatment derives mainly from the electricity used for lamp functioning.

However, doping may extend the light absorption in the range of long UV and visible wavelengths allowing the process to be activated by sunlight. Solar light-activated photocatalysis could be an economical and sustainable technique to treat polluted water streams.

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Typical dopants are noble metals as Pt, Ag, Cu, Ni, Rh, Pd (*Kumar & Rao, 2017; Nasirian & Mehrvar, 2016; Ofiarska et al., 2016*) or non-metals such as N, C, F or S (*Fujishima et al., 2008*). Among the two doping techniques, doping with non-metal compounds is economically convenient compared to noble metal doping.

To the best of author's knowledge applications of titanium dioxide photocatalysis for HWW and more in detail for cytotoxic compounds exist only at the lab scale.

In Table 2.4 some studies about the application of TiO₂ photocatalysis are reported.

It is useful to underline that all the studies achieved good results but only at laboratory controlled conditions.

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Table 2.4: Review of studies about photocatalytic oxidation of cytotoxic compounds

Cyt	Catalyst	Matrix	pH	Cat load (mg/L)	Conc (mg/L)	Light intensity	λ (nm)	T (°C)	k _{app} (min ⁻¹)	T _{irr} (min)	η _{deg} (%)	Ref.
5-FU	Degussa P25	PW ⁽¹⁾	5.8	20	0.2	8 W	254	25±2	0.0375	90	99.9	[1]
CP			5.8	20	0.1				0.0434			
IF	Bi-B-TiO ₂	PW ⁽¹⁾	7	250	5	430 W/m ²	AS ⁽³⁾ : >290 VS ⁽⁴⁾ : >420	25±2	0.0240 (AS ⁽³⁾)	90	100	[2]
	Pure TiO ₂			2500	50				0.0180 (VS ⁽⁴⁾)			
5-FU	N-doped TiO ₂	PW ⁽¹⁾	5.6	20	0.2	16 W	BL ⁽⁵⁾ : 390-500	25±2	-	1200	88.8	
CP	TiO ₂								-		59.3	
5-FU	Degussa P25								-		61.5	
CP									-		-	

[1] Lin & Lin, 2014; [2] Fiszka Borzyszkowska et al., 2016; [3] Lin, Lin, & Hung, 2014;

The meaning of the symbols for cytotoxic compounds is the following:

5-FU= 5-fluoracil; CP= cyclophosphamide; IF= ifofosfamide; MTX = methotrexate; DR=doxorubicin

The meaning of the other symbols are:

(1) PW = pure water (mQ water); (2)DW = demineralized water; (3) AS=artificial sunlight; (4) VS = visible light; (5) BL = blue light;

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Table 2.4: Review of studies about photocatalytic oxidation of cytotoxic compounds

Cyt	Catalyst	Matrix	pH	Cat load (mg/L)	Conc (mg/L)	Light intensity	λ (nm)	T (°C)	k _{app} (min ⁻¹)	T _{irr} (min)	η _{deg} (%)	Ref.
IF	Pt-TiO ₂	PW ⁽¹⁾	7	500	5	550 W/m ²	AS ⁽³⁾ : >290 VS ⁽⁴⁾ : >420	25	0.294 (AS ⁽³⁾)	60	100	[4]
	Pure TiO ₂		5.5	5000	50				0.0148 (AS ⁽³⁾) 0.0093 (VS ⁽⁴⁾)	240 >240	100 ≈ 90	
	Pt-TiO ₂		5.5	5000	50				0.0783 (AS ⁽³⁾)	60	100	
Pure TiO ₂	0.0181 (AS ⁽³⁾)	240										
IF	Degussa P25	DW ⁽²⁾	5.5	100	0.1	8 W	352	25	0.433±0,024	10	100	
MTX	Degussa P25	DW ⁽²⁾	6.4	500	20	2057 mJ/cm ²	200-440	25±2	0.234	4	82	[5]
MTX	Degussa P25	DW ⁽²⁾	-	200	15	40 W	λ _{max} = 390	26	t _{1/2} = 2 min	30	100	[6]

[4] Lai et al., 2015; [5] Lutterbeck et al., 2015; [6] Calza et al., 2014

The meaning of the symbols is the following:

5-FU= 5-fluoracil; CP= cyclophosphamide; IF= ifofosfamide; MTX = methotrexate; DR=doxorubicin

(1) PW = pure water (mQ water); (2) DW = demineralized water; (3) AS=artificial sunlight; (4) VS = visible light; (5) BL = blue light

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The values of the k_{app} in Table 2.4 refers to the pseudo-first order constant of degradation of the cytotoxic compounds.

A pseudo-first order kinetic describes a reaction involving two reactants where the concentration of one of them remains constant during the process. In the case of the photocatalysis of cytotoxic compounds, it is the TiO₂ concentration that is stable while the concentration of the cytotoxic drug decreases. This is a good approximation of reality because TiO₂ concentration is typically five or six orders of magnitude higher than the one of the cytotoxic compounds and photocatalyst remains substantially unchanged in the process if no adsorption establishes between the two compounds. So, the rate of the reaction can be considered to be first order even if the reaction involves two reactants. If the process can be simplified through React. 2.7:



where Cyt represent the cytotoxic compound, TiO_2 the titanium dioxide concentration and TPs are the reaction transformation products.

The rate law r (s⁻¹) can be calculated according to Eq. 2.4.

$$r = k[TiO_2][Cyt] = -\frac{d[Cyt]}{dt} \quad \text{Eq. 2.4}$$

where:

- k = reaction rate coefficient (L s mol⁻¹);
- $[TiO_2]$ = TiO₂ concentration (mol L⁻¹);
- $[Cyt]$ = cytotoxic compound concentration (mol L⁻¹);

As the concentration of the photocatalyst is supposed not to change during the process it is possible to group the reaction rate coefficient r and TiO₂ in one coefficient called the *apparent rate coefficient* or the *apparent constant of degradation* k_{app} (Eq. 2.5). k_{app} has dimension of the inverse of time.

$$r = (k[TiO_2])[Cyt] = k_{app}[Cyt] = -\frac{d[Cyt]}{dt} \quad \text{Eq. 2.5}$$

2. STATE OF THE ART

Electrochemical oxidation

Electrochemical oxidation exploits the generation of electricity with electrodes to dissociate organic molecules. In particular, pollutants may be directly oxidised by electron transfer with the anode (“anodic oxidation”) or attacked by radicals (mainly hydroxyl radicals) generated during the process (“mediated oxidation”). The dosage of chlorine facilitate the degradation of the target contaminants through the generation of active chlorine species like hypochlorite, chlorine dioxide and chlorine.

Regarding mediated oxidation, the first step of the process is formation of OH· radicals at the surface of the anode.

After the first step the degradation pathway depends on the nature of the electrodes that can be distinguished in “active” or “non-active” electrodes.

Active electrodes promote the formation of oxides thanks to the interaction between the adsorbed OH· and the anode surface.

The oxide, known as “active oxygen”, can attack organic contaminants, such as cytotoxic compounds, leading to their oxidation.

Commonly used active electrodes are stable electrodes such as Pt and Ti electrodes whose surface can be oxidised to form active oxygen.

On the contrary, non-active electrodes are made of materials as boron-doped diamond (BDD) or oxides of Sn or Pb that cannot be furtherly oxidised. So, adsorbed hydroxyl radicals do not react with the surface forming active oxygen but directly oxidise organic compounds in solution.

Non-active electrodes leads to demineralization of the target compounds while active electrodes leads to oxidised by-products whose effects in term of ecotoxicity should be investigated in order to fully understand the efficacy of the process.

Applications of electrolysis of cytotoxic compounds are reported in Table 2.5.

In particular, in a study by Fabianska and other authors (*Fabiańska et al., 2015*) it is underlined that the presence of other ions such as Cl⁻, PO₄³⁻, NH₄⁺, NO₃⁻ and HCO₃⁻ may even enhance the process due to the formation of other radicals species with higher oxidant power or selectivity than hydroxyl radicals. Thinking about possible applications with real HWWs this is encouraging as HWWs contain many different

2. STATE OF THE ART

ionic species. In fact, good results with hospital wastewater or human urine were obtained by others (*Kobayashi et al., 2008, 2012*).

Table 2.5: Electrolysis of cytotoxic compounds

Treatment	Compounds	Matrix	Results	Ref
Electrolysis at BDD anode	CP, IF	Pure water	$\eta_{deg}(\%) \approx 97$	[1]
Electrolysis at Pt based Ir anode	Mix of compounds (MTX, CF, GEM, 5-FU...)	Model wastewater (12 compounds) Clinical wastewater (5-18 compounds)	<u>Reduction of measured cytotoxicity:</u> 99.6 % (model wastewater) 94.9 ± 7.7% (clinical wastewater)	[2]
	13 compounds (MTX, CP...)	Single-compound solutions + NaCl Solution containing a mix of 13 contaminants + NaCl	<u>Drug inactivation</u> Single compounds: (cytotoxic activity) _{final} = 1/3-1/100 000 (cytotoxic activity) _{initial} Mix of contaminants: (cytotoxic activity) _{final} = 1/100 (cytotoxic activity) _{initial}	[3]
	MTX	Human urine + 153 mM NaCl	$\eta_{deg}(\%) > 95$	[4]

[1] *Fabiańska et al., 2015*; [2] *Kobayashi et al., 2008*; [3] *Hirose et al., 2005*; [4] *Kobayashi et al., 2012*.

2. STATE OF THE ART

Fenton and photo-Fenton process

Fenton process exploits the reaction between hydrogen peroxide and iron ions to produce hydroxyl radicals. Fe²⁺ ions act as catalysts in H₂O₂ decomposition initiating a complex reaction sequence (*Ganiyu et al., 2015*).

In the case of photo-Fenton oxidation, UV or UV/vis ($\lambda < 400$ nm) light is used to catalyse Fenton reactions through photo-reduction of Fe³⁺ and decomposition of Fe(II) hydroxide enhancing productions of OH[·]. In fact, at pH=3.5 Fe is mainly present in the form of Fe(OH)²⁺ which has a strong absorption in the UV region.

The Fenton process is optimal at acid pH in the range 2.5 < pH < 3.5 as at pH > 3.5 the Fe³⁺ ions precipitate in the form of Fe(OH) and at pH < 2.5 the production of hydroxyl radicals becomes difficult (*Ganiyu et al., 2015*). Thus, the process seems not economically convenient for HWW (typical pH=7-8) as a correction of pH is required.

Few applications regarding HWW or pharmaceuticals-contaminated water with Fenton process exist.

To the best of author's knowledge, up to now only one study have been performed to treat cytotoxic compounds.

A study on Fenton and photo-Fenton degradation of 5-fluoracil proved that it was possible to degrade the compound with this technique achieving respectively 50% and 67% mineralization yields after 8 h of treatment. The procedure was tested on a real WWTP effluent spiking 5-fluoracil achieving even higher degradation rate compared to degradation in synthetic water (*Governo et al., 2017*).

Anand et al. tested a solar photo-Fenton reactor to treat HWW and managed to reduce the COD by 98% and increase the BOD₅/COD ratio from 0.16 to 0.7 demonstrating the improved biodegradability of the treated water (*Anand et al., 2016*).

3 MATERIALS AND METHODS

The experimental methodology followed the scheme adopted by other authors studying photocatalysis of cytotoxic compounds (among them: *Lai et al., 2015; Lin & Lin, 2014; Ofiarska et al., 2016*). In particular, before evaluating the photocatalysis of the studied compound, some experiments were performed to evaluate the adsorption of the compounds in the dark and their capability to photodegrade in absence of the photocatalyst. These experiments are necessary to isolate the contribution of the non-photocatalytic phenomena to *cytotoxics* degradation.

In the present work 3 cytotoxic compounds were selected: methotrexate (MTX), gemcitabine (GEM) and cyclophosphamide (CP). MTX was detectable by both spectrophotometry and High Performance Liquid Chromatography (HPLC) as GEM and CP only by HPLC.

MTX was chosen as sample compound to optimize the procedure since it was possible to measure its degradation with the spectrophotometer; in fact, the compound is in the form of a yellow powder that gives a yellowish colour to the medium. However, a first experiment was performed measuring MTX concentration both with spectrophotometry and HPLC to assess if the two analytical techniques give comparable results.

The effect of the main parameters influencing the photocatalysis, namely pH, TiO₂ load, MTX concentration and irradiation wavelength, was studied to optimize the photocatalysis of this compound. When one parameter was varied, the other ones were kept constant to ensure comparability between the experiments.

All these experiments were performed in pure water and the solution was irradiated for 60 minutes. Irradiation was provided by a low pressure UV lamp except for experiments on the effect of the irradiation wavelength when a medium pressure UV lamp was tested too. As it was not possible to replicate all the experiments for time

3. MATERIALS AND METHODS

reasons, experiments were repeated only for one value in the range of variability of each studied parameter.

In the end, one experiment on MTX photocatalysis was performed in synthetic urine to assess if the technique is efficient in a more complex matrix. In this case, it was necessary to work at higher concentrations than in pure water ([MTX]=10 mg/L) to ensure MTX detectability by spectrophotometry.

Afterwards, the optimized procedure was tested on a mix of MTX, GEM and CP in order to understand the influence between the compounds and the possibility to degrade them at the same optimal conditions found for methotrexate. As HPLC was used to measure the concentration of the compounds, it was possible to study degradation at lower concentrations ([MTX]=[GEM]=[CP]=100 µg/L). The effect of initial pH on the degradation of the mixture was studied. The framework of the experiments tested values of each parameter is shown in Figure 3.1.

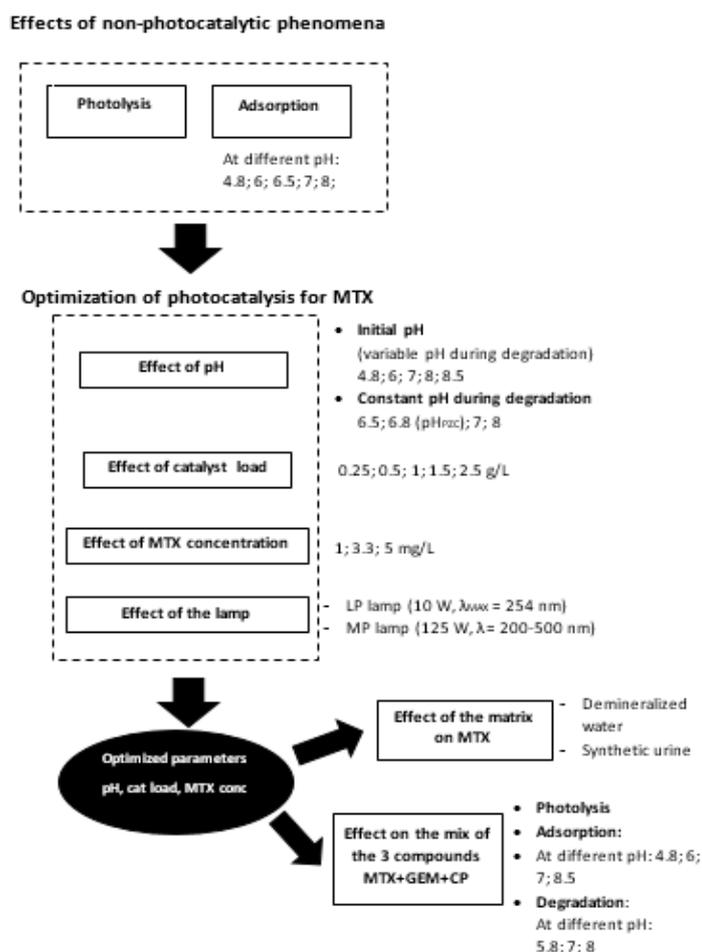


Figure 3.1: Experimental framework

3. MATERIALS AND METHODS

3.1 Chemicals

The three cytotoxic compounds, MTX, CP and GEM, were provided by Sigma Aldrich. Stock solutions were prepared diluting the pharmaceuticals in a solution of mQ water (resistivity 18.2 MΩ·cm) and methanol (methanol concentration=1mg/mL) and then stored in dark at -20°C.

Aeroxide® TiO₂ P25 (Evonik, Germany) was the photocatalyst used in a slurry phase during photocatalytic experiments; its physico-chemical characteristics and chemical composition are summarized in Table 3.1.

Table 3.1: Physico-chemical properties of Aeroxide® P25 TiO₂

www.aerosil.com/www2/uploads/productfinder/AEROXIDE-TiO2-P-25-EN.pdf

Properties	Unit	Value
Specific surface area (BET)	m ² /g	35-65
pH value in 4% dispersion	-	3.5-4.5
Loss on drying (2 h at 105°C)	%	≤ 1.5
Tamped density	g/l	100-180
Sieve residue (by Mocker, 45µm)	%	≤ 0.050
TiO ₂ content	%	≥ 99.50
Al ₂ O ₃ content	%	≤ 0.300
SiO ₂ content	%	≤ 0.200
Fe ₂ O ₃ content	%	≤ 0.010
HCl content	%	≤ 0.300

The photocatalyst was stored in the dark at ambient temperature to avoid accidental photoactivation.

The synthetic urine solution was prepared according to Method 6301.1 suggested by the Center for Disease Control and Prevention (CDC, 2010). The procedure involves the mix in sequence of the following reagents before adding 1 L of demineralized water. The reagents and their quantities are:

- 500 mL of demineralized water;
- 3.8 g of potassium chloride (KCl);
- 24.5 g of urea (CO(NH₂)₂);

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- 1.03 g of citric acid (C₆H₈O₇);
- 0.34 g of ascorbic acid (C₆H₈O₆);
- 1.18 g of potassium phosphate (KH₂PO₄);
- 1.4 g of creatine (C₄H₉N₃O₂);
- 0.64 g of sodium hydroxide (NaOH) to add slowly;
- 0.47 g of sodium bicarbonate (NaHCO₃);
- 0.28 mL of sulfuric acid (H₂SO₄);

The prepared solution must be stored in the refrigerator at 4°C.

The composition of synthetic urine is reported in Table 3.2.

Table 3.2: Synthetic urine composition

Compound	Formula	M (moles/L)
Potassium chloride	KCl	5.1×10^{-2}
Urea	CO(NH ₂) ₂	4.08×10^{-1}
Citric acid	C ₆ H ₈ O ₇	5.36×10^{-3}
Ascorbic acid	C ₆ H ₈ O ₆	1.93×10^{-3}
Potassium phosphate	KH ₂ PO ₄	8.54×10^{-3}
Creatine	C ₄ H ₉ N ₃ O ₂	1.07×10^{-2}
Sodium hydroxide	NaOH	1.60×10^{-2}
Sodium bicarbonate	NaHCO ₃	5.6×10^{-3}
Sulfuric acid	H ₂ SO ₄	2.8×10^{-4}

3.2 Photocatalytic experiments

The set-up consisted in a 1.5 L glass batch reactor containing a UV lamp centrally-located, as show in Figure 3.2 and Figure 3.3. The surface of the reaction vessel was covered by an aluminium foil in order to screen it from outside irradiation and reflect radiation maximizing photocatalyst illumination. Shape and dimensions of the reaction vessel are reported in Figure 3.3.

The reaction vessel was connected to a cooling system (LAUDA Microcool MC250) to ensure that the temperature of the reaction was stable.

A magnetic stirrer guaranteed adequate stirring of the medium in the reactor; the stirring rate was set at 550 rpm.

3. MATERIALS AND METHODS

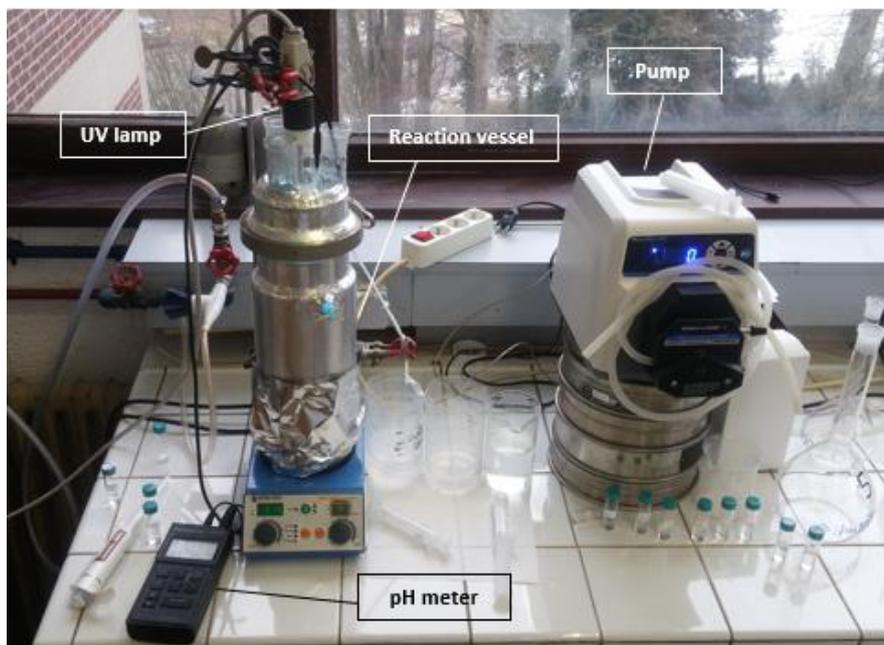


Figure 3.2: Experimental set-up

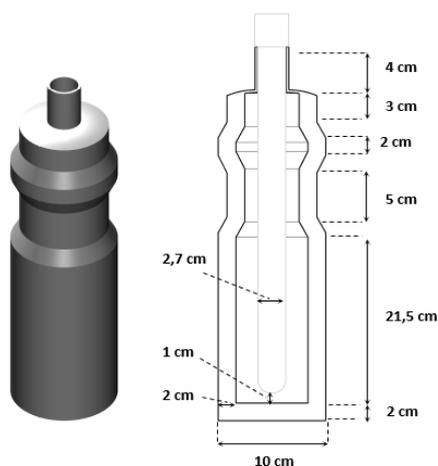


Figure 3.3: Schematic representation of the reaction vessel

Two different Hg vapours UV lamp (Helios Ital quartz, Italy) were tested. The two types differ for the pressure of the Hg vapours that surround the lamps. Namely, we tested:

- a *low pressure UV-C germicide lamp* (10 W) with a monochromatic spectra concentrated around 254 nm;
- a *medium pressure UV-A lamp* (125 W) with a broader spectra in the UV region. The emission spectra of the lamp is reported in Figure 3.4.

3. MATERIALS AND METHODS

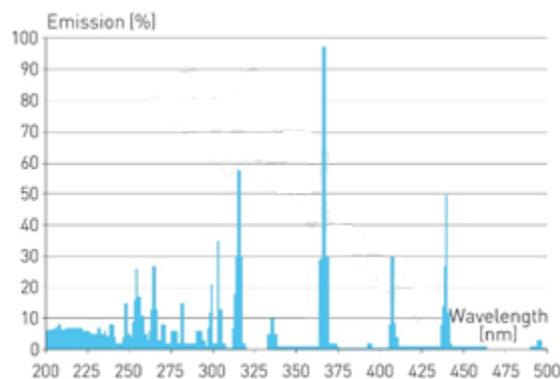


Figure 3.4: Irradiation spectrum of the medium pressure UV lamp provided by the supplier

(www.heliosquartz.com/prodotti/lampade-uv-a-media-pressione)

Lamps were placed in a transparent quartz sleeve to protect them from abrasion.

3.3 Experimental procedure

The reactor was cleaned three times with 1.5 L of demineralized water×time and one time with 1.5 L of mQ water. HPLC and spectrophotometry analysis showed that negligible mass of contaminants remained after the four cleaning steps. A pump was used to empty every volume of cleaning water.

All the solutions were prepared spiking 1 L of the medium in the reaction vessel with the desired amount of cytotoxic compound tipped from the stock solution. When different cytotoxic compounds were dosed the tip of the pipette was changed for every compound.

Solutions were stirred 10 minutes before sampling to obtain homogeneous concentration in the reactor.

The desired amount of TiO₂ was weighted with a balance (precision=0.01 g) before adding it into the reactor.

The photocatalyst was dosed in dispersion with additional 0.5 L of the same medium in the reactor.

When required pH was adjusted tipping NaOH from two different solutions at 0.2 and 0.02 M.

Before switching on the lamp and start the photocatalysis the solution was stirred in the dark at least 30 minutes to reach the adsorption equilibrium at stable pH.

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Then, the UV lamp was switched on to start the photodegradation of the cytotoxic compound. Samples were taken at fixed time steps to follow the degradation.

Every sample was filtered through a 0.2 µm cellulose filter to block TiO₂ particles and was stored in the dark at -20°C before analysis. Sampling procedure is explained in more detail in Appendix A.

Temperature and pH were monitored during all the experiments. Temperature was set at 19±1°C while pH varied during degradation.

3.3.1 Adsorption quantification

Adsorption of the compounds was studied at different pH and quantified with two quantities: *adsorption ratio* (%) and *specific adsorption* (mg_{Cyt}/g_{TiO₂}).

The adsorption ratio, *Ads*, is the ration between the adsorbed and the dosed mass:

$$Ads(\%) = \frac{M_0 - M_1}{M_0} \times 100 = \frac{(C_0 \times V_0) - (C_1 \times V_1)}{(C_0 \times V_0)} \times 100 \quad \text{Eq. 3.1}$$

The specific adsorption, *Ads_{sp}*, instead, is the ratio between the adsorbed mass (mg_{Cyt}) and the dosed mass of TiO₂ (g_{TiO₂}):

$$Ads_{sp}(mg_{MTX}/g_{TiO_2}) = \frac{(C_0 \times V_0) - (C_1 \times V_1)}{M_{TiO_2}} \quad \text{Eq. 3.2}$$

The meaning of the variables in Eq. 3.1 and 3.2 is the following:

- C_0 = initial *cytotoxic* concentration (mg/L)
- M_0 = Initial *cytotoxic* mass in solution (mg) = $C_0 \times V_0$
- C_1 = *cytotoxic* concentration after adsorption (mg/L)
- M_1 = *cytotoxic* mass after adsorption (mg)
- V_0 = initial volume without photocatalyst (L)
- V_1 = volume after photocatalyst addition (L)
- M_{TiO_2} = photocatalyst load (g)

The initial concentration C_0 and the cytotoxic concentration after adsorption C_1 are the mean value obtained by three samples.

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3.3.2 Kinetic constant evaluation

The photocatalytic process was modelled with a *pseudo-first order kinetic* since it properly fits the concentration decrease during photocatalytic processes (*Fiszka Borzyszkowska et al., 2016; Lai, Lin, & Lin, 2015; Lutterbeck et al., 2015; Ofiarska et al., 2016* among those who worked on cytotoxics).

Modelling the process with a first order kinetics allows the calculation of the apparent reaction rate with a simple linearization. In fact, the integration of the Eq. 3.3 between time 0 and the generic time t leads to Eq. 3.4.

$$[Cyt]_t = [Cyt]_0 \exp(-k_{app} t) \quad \text{Eq. 3.3}$$

$$\ln[Cyt]_t = \ln[Cyt]_0 - k_{app} t \quad \text{Eq. 3.4}$$

where $[Cyt]_0$ is the cytotoxic concentration right before irradiation, t is time and $[Cyt]$ is the concentration of the cytotoxic at the generic time t .

Excel was used to estimate the k_{app} calculated using the function “Add trendline” whose outputs are the equation of the trendline and the R² coefficient. The more R² value is closer to 1 the better the trendline fits the data.

The intercept with the y-axis was set equal to the measured $[Cyt]_0$ for each set of experimental data.

It is important to underline that in the case of spectrophotometry analysis the calculated value of the k_{app} do not refers to rate of degradation of the parent compound but it is a quantity that refers to the degradation rate of the mixture of the parent compound and transformation products in the matrix.

3.4 Analytical methods

Two different techniques were used to measure the concentration of the cytotoxic compounds: spectrophotometry and High Performance Liquid Chromatography coupled Mass Spectrometry (HPLC/MS).

Spectrophotometry was used to measure methotrexate and HPLC/MS was used to measure the concentration of the three compounds in a mixture.

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Spectrophotometry analysis

A HACH DR 4000 U spectrophotometer was used to analyse methotrexate concentration in solution. The maximum peak of absorption of methotrexate was detected at 300 nm (ABS=0.171 in comparison to a blank sample, Figure 3.5)

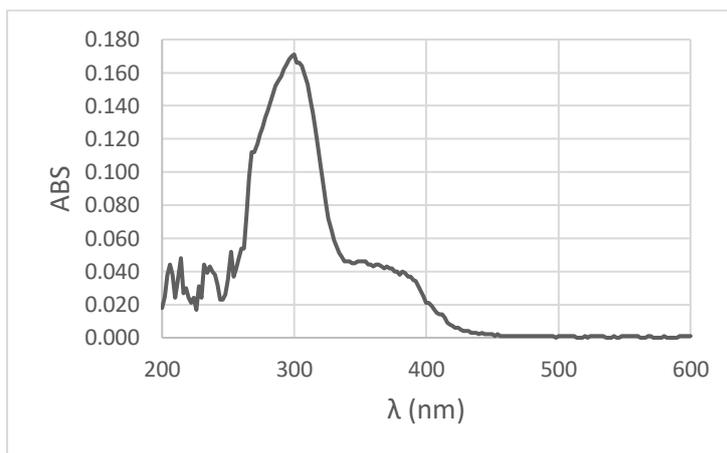


Figure 3.5: MTX absorption spectre in ultrapure water ([MTX]=5 mg/L).

The Limit of Detection (LOD) of the instrument is ABS=0.002 (3 times the standard deviations of the ABS values given by blank samples) and the Limit of Quantification (LOQ), estimated as 10 times the standard deviation of the values registered for blank samples, is 0.006. Anyway, samples whose ABS was lower than 0.010 were not considered to reduce the experimental error.

A calibration curve was made measuring samples at concentrations: 0.1 – 0.5 – 1 – 2.5 – 5 – 7.5 – 10 mg/L. For each concentration, three different samples were prepared to measure absorption and assess the variability of the absorption measures. The direct relationship between absorption at 300 nm and concentration is showed in Figure 3.6.

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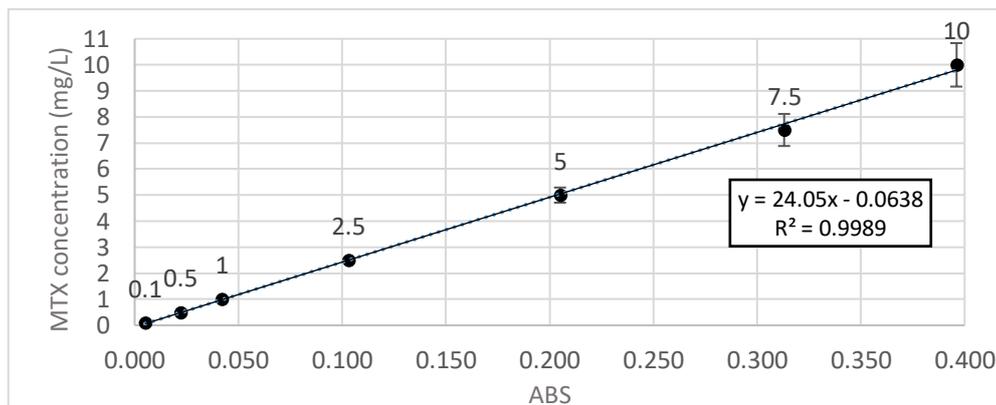


Figure 3.6: Calibration curve for MTX in pure water. Error bars represents the standard deviation of the concentration. The values over each point are the concentration values used to calibrate the instrument.

The relationship between ABS and MTX concentration is linear ($R^2=0.999$) and it is expressed by Eq. 3.5.

$$[MTX] = 24.905 \cdot ABS - 0.0638 \quad (R^2=0.9989) \quad \text{Eq. 3.5}$$

The same procedure as the one for pure water was followed to calibrate spectrophotometry measures in synthetic urine. The maximum absorption peak was still at 300 nm ($ABS=2.563$). The instrument was calibrated at concentration values=5-7.5-10 mg/L. It was necessary to work at higher concentrations since MTX was not detectable for concentration lower than 5 mg/L. The calibration curve was still a straight line ($R^2=0.9989$) and it is plotted in Figure 3.7.

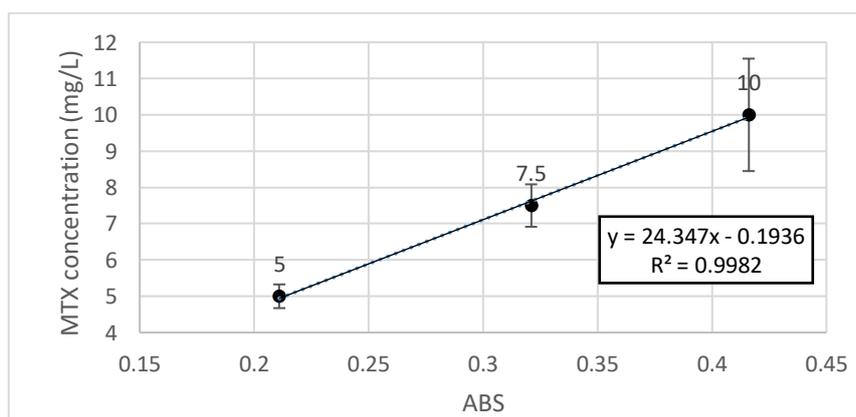


Figure 3.7: Calibration curve for MTX in synthetic urine. Error bars represents the standard deviation of MTX concentration. The values over each point are the concentration values used to calibrate the instrument.

3. MATERIALS AND METHODS

The relationship between measured ABS and MTX concentration is expressed by Eq. 3.6.

$$[MTX] = 24.347 \cdot ABS - 0.1936 \quad (R^2=0.9982) \quad \text{Eq. 3.6}$$

HPLC/MS analysis

HPLC/MS analyses were performed through an Agilent 6495 triple quadrupole LC/MS apparatus.

The LC apparatus works extracting 10 µL from a sample vial filled during an experiment. A syringe extracts the sample volume at a speed of 8 µL /s. Each sample volume is pumped at 10 bar with an Accela 1250 pump through the elution column. The elution column (KINETEX C18) is made of packed silica grains and its dimensions are 150x2.1 mm (cylindrical column, 150 mm length, 2.1 mm diameter). The HPLC set-up can be classified as Reversed-Phase Chromatography (RPC). Contrary to Normal-Phase Chromatography, that employs unmodified silica resins as hydrophilic stationary phase, RPC uses a hydrophobic stationary phase obtained bonding an octadecyl carbon chain (C18) to silica. The temperature of the column is kept at 30°C. The mobile phase was obtained from a mixture of two different solvents: solvent A (98.9% water, 1% CH₃CN and 0.1% HCOOH) and solvent B (CH₃CN). The composition of the mobile phase varied during the process to facilitate the elution of the three cytotoxic compounds (see Table 3.3). The mobile phase flowrate was 200 µL/min.

Table 3.3: Mobile phase composition

Mobile phase composition		
Time (min)	Solvent A content (%)	Solvent B content (%)
	Solvent A composition: 98.1 % H ₂ O, 1% CH ₃ CN, 0.1% HCOOH	Solvent B composition: 100% CH ₃ CN
0	97	3
30	3	97
32	97	3
32	97	3

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The mass spectrometer was run in positive mode. Mass-to-charge ratios (m/z) used by the mass spectrometer to detect the three cytotoxic compounds are reported in Table 3.4.

Table 3.4: m/z ratios of the studied compounds

Compound	m/z ratio
MTX	455.2
GEM	261.0
CP	264.1

4 RESULTS

4.1 Analytical results

MTX concentrations obtained by spectrophotometry and HPLC are reported in Figure 4.1.

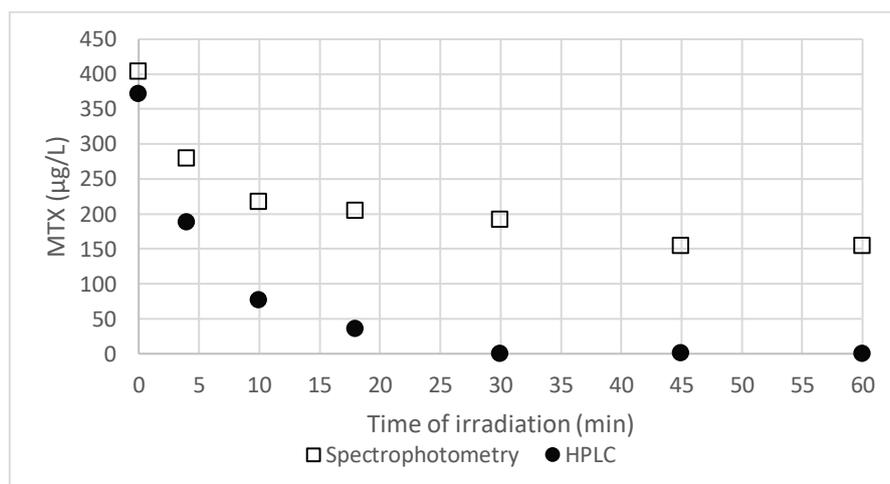


Figure 4.1: MTX concentration vs time, measure by HPLC and spectrophotometry
[MTX]=400 mg/L, [TiO₂]=0.5 g/L, pH=7. 1 LP UV lamp (10 W, 254 nm)

HPLC and spectrophotometry start to detect different concentrations few minutes after degradation. This demonstrates that TPs originate quickly and can absorb light at 300 nm. In fact, after 30 minutes HPLC measures null concentration while the spectrophotometer still reveal the presence of the compound.

In some experiments (see section 4.3.3), MTX disappeared in 10-15 minutes as reported by Calza (*Calza et al., 2014*).

TPs generated by the photocatalysis of MTX with TiO₂ have been recognized by others (*Lutterbeck et al., 2015*, see Figure 4.2).

4. RESULTS

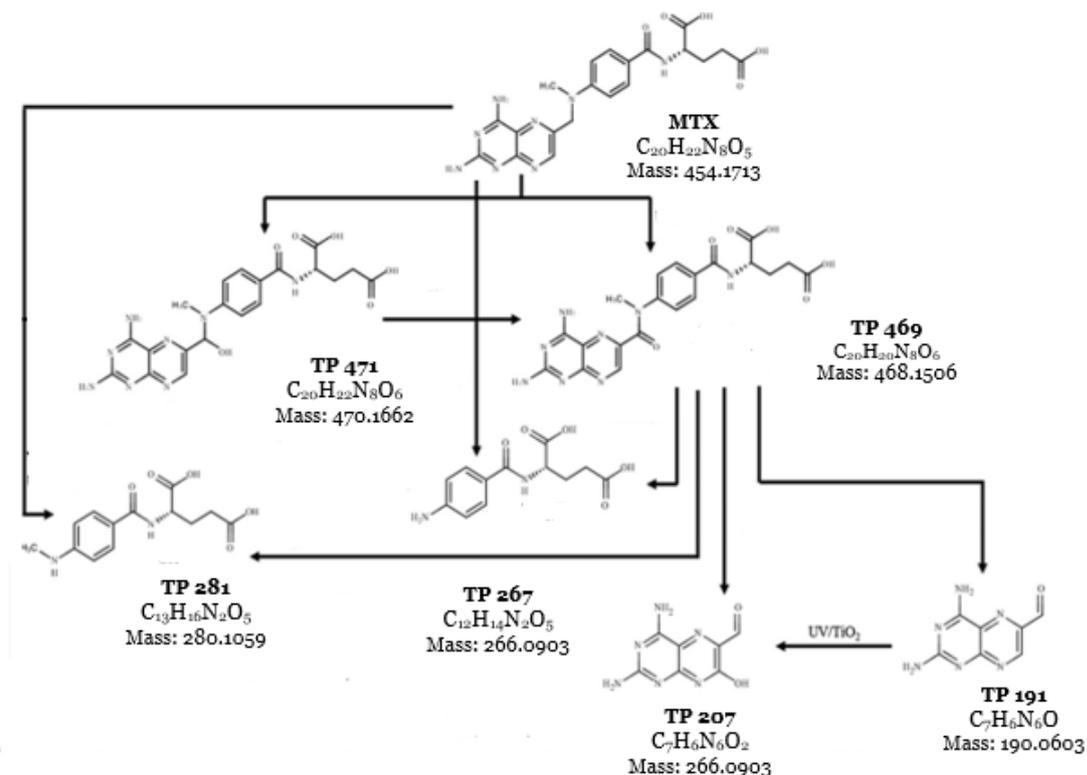


Figure 4.2: Transformation products of MTX with UV/TiO₂ treatment

(adapted from *Lutterbeck et al., 2015*)

Differences in concentration measures reflect into different values of the constant of degradation. Kinetic calculations made on spectrophotometry data lead to lower values of the degradation constant ($k_{\text{spectro}}=0.066 \text{ min}^{-1} \ll k_{\text{HPLC}} = 0.209 \text{ min}^{-1}$ for experiment in Figure 4.1) because of the parent compound and MTX by-products.

HPLC is a more precise instrument that allows the distinction of the parent compound and its TPs but spectrophotometry was useful to have a cheap and fast response on the efficiency of the process.

The decision to use spectrophotometry knowing the limits of the technique (limit of detection, difficulty in distinguishing between the parent compound and its TPs) is acceptable because this work configures as a preliminary study in a more complex project that is at the very beginning.

The final goal of the project is to couple the photocatalytic reactor with a membrane to recover TiO₂ particles (PMR, Photo Membrane Reactor); this PMR will be tested at

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the pilot scale with real hospital wastewaters and the parameter to assess the efficacy of the set-up will be the decrease of toxicity.

The decrease in toxicity is the best parameter to evaluate the environmental performances of the set-up as also the TPs of cytotoxic compounds can be eco-toxic (*Calza et al., 2014*). Measuring the removal efficiency of the parent compound only gives a partial idea on the efficacy of the process. However, eco-toxicity analyses are expensive and time-consuming so they will be implemented only at the end of the project when the procedure will be more consolidated.

HWWs contains a lot of different contaminants but it was decided to treat cytotoxic compounds as the increase in cancer cases worldwide will increase the presence of these contaminants in the environment. Among all the contaminants, they proved to be very persistent and difficult to degrade.

Spectrophotometry was a cheap method to start studying the system as other authors proved that there is a direct relationship between the decrease in absorption at a precise wavelength and the removal of a pharmaceutical compound (*Wittmer et al., 2015*).

For its nature of preliminary study, higher concentration than environmental ones were studied so as the working conditions were far from the limit of detection of the spectrophotometer.

4.2 Photocatalysis of MTX

4.2.1 Adsorption in the dark

The adsorption of MTX was studied at different pH values for the initial MTX concentration of 3.3 mg/L and the same photocatalyst load of 0.5 g/L.

Results are shown in Figure 4.3.

4. RESULTS

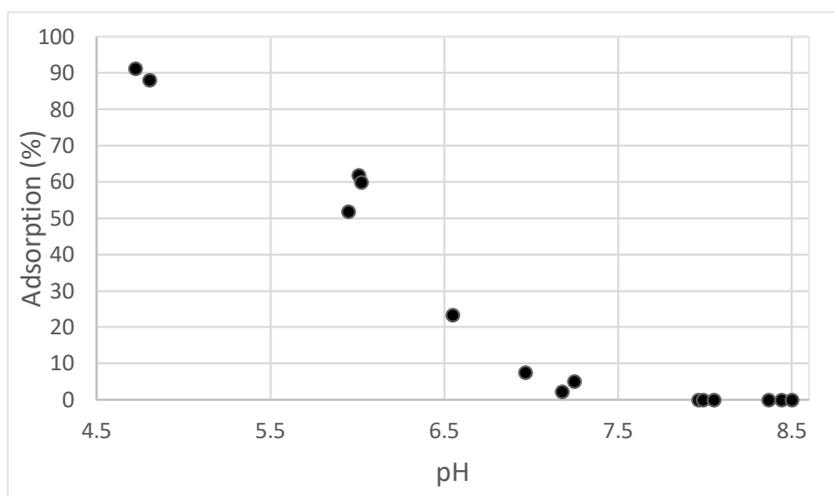


Figure 4.3: Effect of pH on adsorption (%). [MTX]=3.3 mg/L; [TiO₂]=0.5 g/L. pH was adjusted with NaOH and at least 30 minutes were waited to reach the adsorption equilibrium

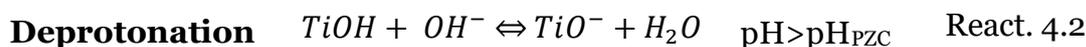
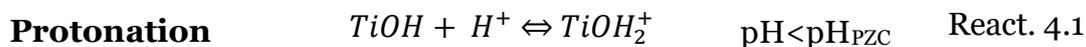
The pH of the system without NaOH addition (MTX and TiO₂ in pure water) was pH≈4.7.

Adsorption decreases for basic pH values being negligible at pH=7.

pH plays a predominant role in electrostatic interactions between TiO₂ and MTX since it determines the surface charge of TiO₂ photocatalyst and the ionization state of both the cytotoxic compound and the photocatalyst.

Degussa P25 TiO₂ (20% anatase and 80% rutile) Point of Zero Charge (pH_{PZC}), namely the pH at which TiO₂ exhibits a neutral charge, is around 6.9 (Kosmulski, 2014) i.e. the surface of photocatalyst presents positive charge if pH<6.9, negative charge otherwise.

Ionization of the surface of the photocatalyst occurs according to Reactions 4.1 and 4.2 (Gad-Allah et al., 2011):



MTX has a pKa ranging from 4.8 to 5.5 (Reid, Yuen, Catolico, & Carlson, 1993). Drugbank.com, a commonly used database for pharmaceutical data (<https://www.drugbank.ca/drugs/DB00563>) reports a pKa value of 4.7. So MTX tends to be deprotonated at pH>4.8 and protonated otherwise.

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Thus, the pH can strongly influence the interaction between MTX and the photocatalyst surface determining attraction or repulsion between TiO₂ and the chemical drug. If attraction occurs cytotoxic molecules can be more easily drawn to the surface of titanium dioxide particles where they can be adsorbed. The relationship between pH and electrostatic interaction between MTX and TiO₂ is schematically represented in Figure 4.4.

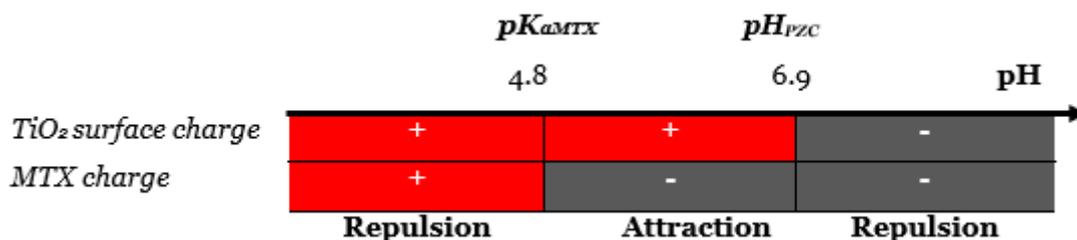


Figure 4.4: Schematic representation of the electrostatic interactions between MTX and TiO₂ surface

In the range between the pH of the system ($\approx 4.7-4.9$ in the presence of TiO₂) and the pH_{PZC} , adsorption is significant and becomes more and more negligible at values around the PZC of titanium dioxide. For $pH > pH_{PZC}$ repulsions should establish between MTX and TiO₂ so no adsorption is observed.

Nevertheless, as adsorption is very high at $pH=4.8$ (88.1-91.2%), it is possible that the MTX in its protonated/non-dissociated form is more affine to TiO₂.

Similar consideration about the effect of pH on electrostatic interactions are described by other authors who studied other cytotoxic compounds as Lin and Lin who worked with 5-fluoracil (*Lin & Lin, 2014*) and Ofiarska and Lai (*Ofiarska et al., 2016; Lai et al., 2015*) who both studied photocatalytic degradation of isofosfamide and cyclophosphamide. Even if they observed that these other cytotoxic drugs did not adsorb on the photocatalyst, they underlined that faster degradation occurred when the cytotoxic compounds and TiO₂ attract because cytotoxic compounds are drawn near the surfaces where oxidation by hydroxyl radicals it is more likely to occur.

Influence of the pH of the solution on the adsorption of the contaminants was studied also by Wang (*Wang & Ku, 2007*) who studied the dependency on pH of dyes adsorption on TiO₂ and by Mrowetz and Selli (*Mrowetz & Selli, 2006*) who obtained similar conclusions working with benzoic acid.

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4.2.2 Photolysis of MTX

One experiment to assess the possibility to photodegrade MTX in absence of TiO₂ was performed at initial MTX concentration of 3.3 mg/L. The initial pH was not adjusted (pH=5). A low pressure UV lamp (10 W, 254 nm) provided irradiation of the water medium for 60 min.

The results are shown in Figure 4.5 and show the possibility to photodegrade MTX even in absence of a photocatalyst.

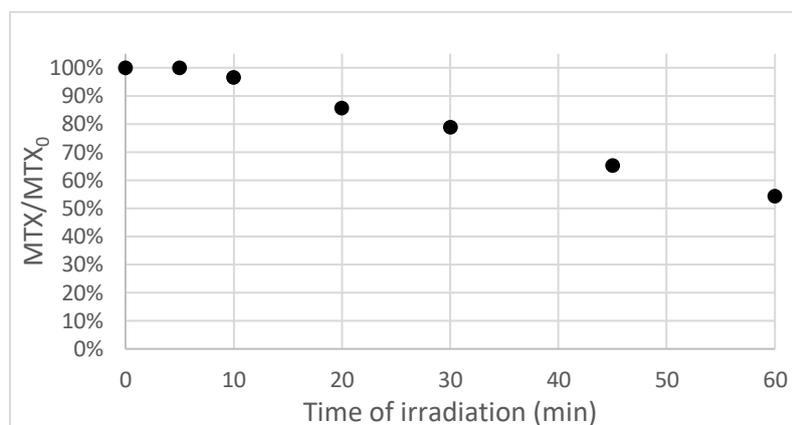


Figure 4.5: Photolysis of MTX. [MTX]=3.3 mg/L, initial pH=5, 1 LP lamp (10 W, 254 nm)

MTX degradation was 44.6% and the apparent constant degradation k_{app} was estimated to be 0.0107 min^{-1} ($R^2=0.9938$).

Other studies found that MTX can undergo direct photolysis. Lutterbeck et al. worked with a medium pressure UV lamp (200-440 nm) showing that MTX parent compound was completely degraded after 128 min but no important demineralization occurred (the DOC removal after 256 min was lower than 5%) (Lutterbeck et al., 2015) as Calza et al. who worked with a 40 W lamp with a maximum emission at 390 nm (60% of degradation after 1 h of irradiation in presence of 0,2 mg/L of TiO₂) (Calza et al., 2014).

4.2.3 Effect of the main parameters: results

After having assessed the effects of adsorption and photolysis the influence of the main parameters regulating the process was studied. All the experimental results are summarized in Table 4.1.

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Table 4.1: Summary of the results (1 LP UV lamp, 60 min of irradiation)

	Ads (%)	k_{app} (min⁻¹)	R²	η_{deg} (%)
Initial pH [MTX]=3,3 mg/L; [TiO ₂]=0,5 g/L				
7	7.6	0.0280	0.9718	79.0
7.2	2.2	0.0291	0.9093	59.6
8	0.0	0.0220	0.8848	70.0
	0.0	0.0213	0.9637	71.8
8.5	0.0	0.0207	0.9568	69.1
	0.0	0.0174	0.9733	75.3
	0.0	0.0217	0.9735	68.2
Stable pH [MTX]=3.3 mg/L; [TiO ₂]=0.5 g/L				
6.8±0.1	5.0	0.0176	0.8412	57.8
7.2±0.1	2.5	0.0156	0.9702	51.2
8±0.1	0.0	-(*)	-(*)	11.9
TiO₂ load (g/L) [MTX] = 3.3 mg/L; pH = 8				
0.25	0.0	0.0191	0.9489	67.5
0.5	2.24	0.0213	0.9637	71.8
	4.99	0.0220	0.8848	70.0
1	0.0	0.0210	0.9093	71.5
1.5	11.5	0.0264	0.7929	68.4
2.5	0.0	0.0254	0.9278	71.9
MTX concentration (mg/L) [TiO ₂] = 0.5 g/L; pH = 8.5				
1	0.0	0.0316	0.9041	82.0
	0.0	0.0267	0.9408	77.2
3.3	0.0	0.0207	0.9568	70.0
	0.0	0.0174	0.9733	75.3
	0.0	0.0217	0.9735	68.2
5	0.0	0.0133	0.8699	64.5
Optimized procedure	7.6	0.0608	0.9727	68.12
[MTX]=1 g/L; [TiO ₂]=0.5 g/L; pH=7	7.4	0.0663	0.9358	66.79

(*) it was not possible to fit a pseudo-first order kinetic

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4.2.4 Effect of pH on photocatalysis

Two sets of experiments were performed to investigate the effect of pH on the photocatalysis of MTX.

The first set of experiments aimed at studying the effect of initial pH: pH was adjusted only at the beginning and then varied during irradiation.

For the second set, the pH was kept constant adding NaOH throughout all the experiment.

The effect of pH was studied in the range from 7 to 8.5 since MTX showed negligible adsorption at this pH values. In fact, MTX adsorption at acidic pH prevents its degradation as probably MTX coverage of TiO₂ surface inhibits its photo-activation as shown by the long experiment at the pH of the system in Figure 4.6 (367 min of irradiation at pH=4.8). As light started some desorption of MTX occurs and the compound is not degraded even after 6 h of irradiation. In this case, adsorption was higher than 90% and then after 20 minutes of irradiation the 10.5% of the adsorbed MTX desorbed.

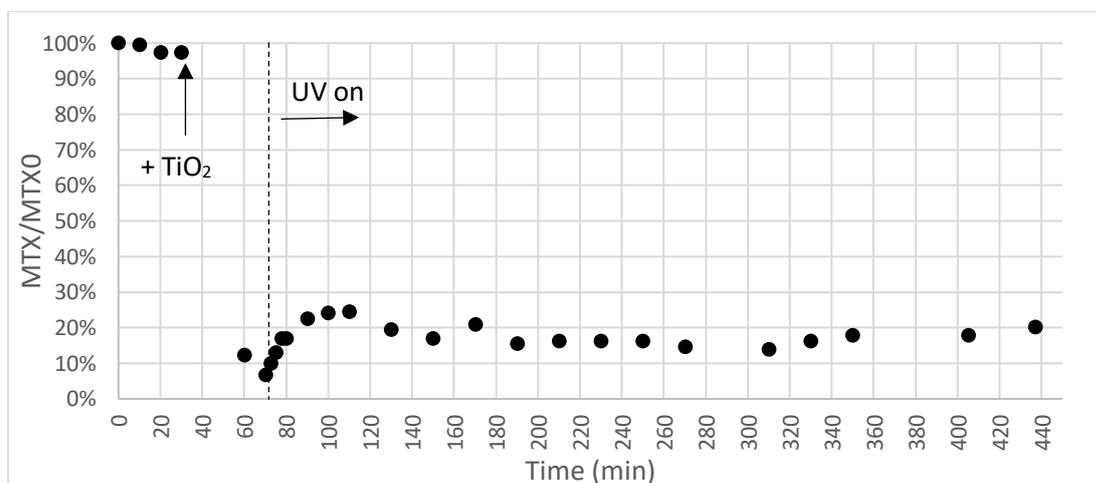


Figure 4.6: Photocatalytic experiment at the natural pH of the system.
[MTX]=3.3 mg/L, [TiO₂]=0.5 g/L, pH = 4.7, 1 LP UV lamp (10 W, 254 nm).
Photocatalyst was added at 35 min and UV started at 70 min.

On the contrary, MTX was degraded during experiments at neutral or basic pH.

The results are shown in Figure 4.7 and Table 4.1.

4. RESULTS

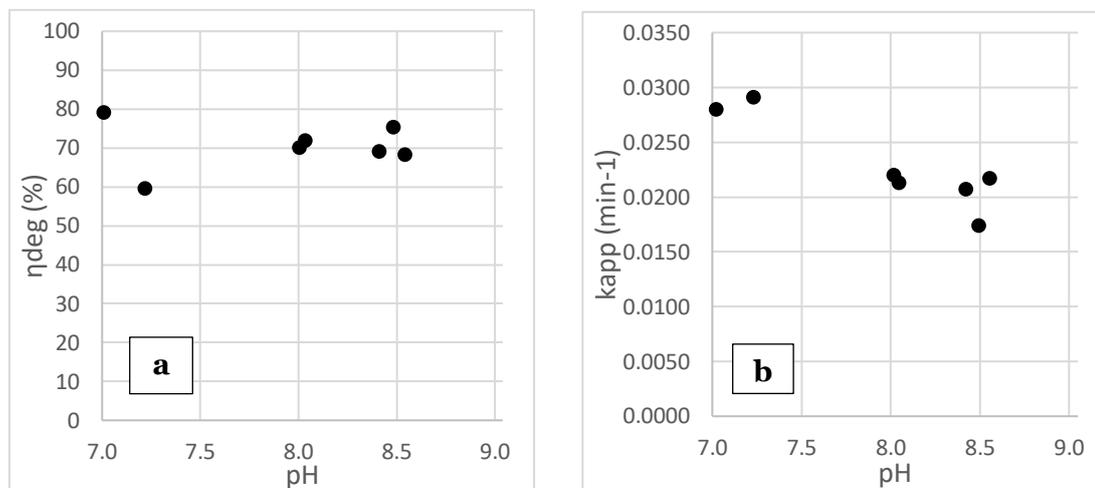


Figure 4.7: Degradation efficiency (a) and rate of degradation (b) as a function of pH. [MTX]=3.3 mg/L; [TiO₂]=0.5 g/L; 1 LP UV lamp (10 W, 254 nm); Time of irradiation=60 min.

Results showed that the degradation was not so sensible to pH as the η_{deg} ranges from 60 to 79%.

The values of k_{app} ranges from 0.0174 to 0.0291 min⁻¹ with the maximum values for the pH=7 (0.280 min⁻¹) and 7.2 (0.291 min⁻¹). This value of pH was optimal probably both for the absence of adsorption that can inhibits degradation and the neutral charge of the photocatalyst (no repulsion).

As stated in Paragraph 4.2.1 , pH influences the reciprocal charge of MTX and TiO₂ and their electrostatic interactions. Electrostatic attraction determines an expected enhancement of degradation since oxidising reactions mainly occur at the surface of TiO₂ where electron holes and adsorbed OH· radicals can attack cytotoxic molecules in solution.

It is important to work at conditions for which the compound to degrade is drawn near the surface of the photocatalyst (attraction or at least neutral conditions). Other authors proved that cytotoxic compounds degrade with the best k_{app} when they have opposite charge with respect to TiO₂ (Ofiarska *et al.*, 2016; Lai *et al.*, 2015; Lin & Lin, 2014).

Similar conclusions about the effect of pH were obtained working on other pharmaceuticals such as paracetamol (Yang *et al.*, 2008) and diclofenac (Sarasidis *et al.*, 2014).

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However, in the case of MTX, the strong affinity with TiO₂ at acidic pH completely inhibited the degradation due to adsorption.

In fact, in another work on photocatalysis of dyes, other authors proved that adsorption of the target compound had a negative impact on degradation due to the reduction of free active sites for radical production (Neppolian *et al.*, 2002).

pH can also influence the degradation mechanism of cytotoxic compounds as experiments showed that at acidic condition the mainly oxidants are electron-holes since in neutral or alkaline conditions degradation occurs mainly due to hydroxyl radical species (Tunesi and Anderson, 1991). In fact, it is logical that at alkaline conditions, the higher concentration of OH⁻ leads to a higher production of OH[•] radicals and a more probable interaction of MTX with them.

In addition, acidic conditions are not favourable to photocatalysis as TiO₂ tends to agglomerate and have a less surface area available for photon absorption (Mozia, 2010).

As pH has a strong influence on photocatalysis and at low non-adjusted pH degradation does not occur (see Figure 4.6), the pH was kept constant adding NaOH throughout all the experiment since pH decreases with irradiation.

However, it is important to underline that the change of pH was not only connected with MTX degradation since a decrease in pH was observed in the presence of TiO₂ only, as showed in Figure 4.8., suggesting that the decrease of pH is due mainly to OH⁻ consumption due to the photocatalyst activation. This results is in contrast from what observed in another study where the pH was adjusted at value 7 and it did not change after irradiation (Fiszka Borzyszkowska *et al.*, 2016).

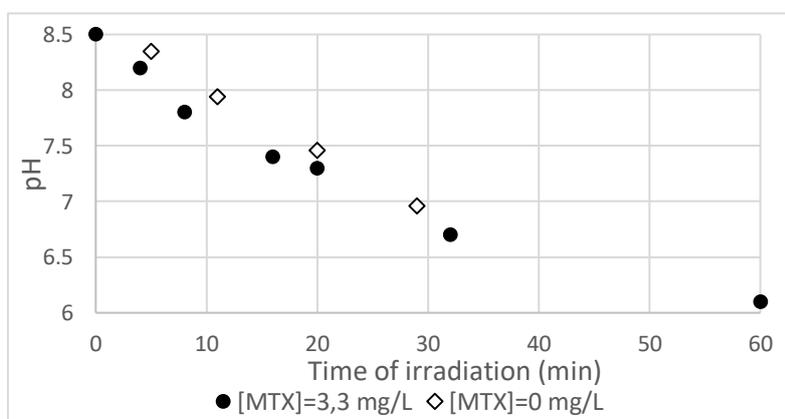


Figure 4.8: Decrease of pH due to irradiation. [TiO₂]=0.5 g/L; initial pH=8.5

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In spite of the expected enhancement of the degradation, experiments at stable pH showed lower and slower degradation (Figure 4.9 and Table 4.1). The worst results were obtained at pH=8 at which degradation was very low (<12%).

The supply of NaOH during irradiation is detrimental to degradation probably because OH⁻ do not have the time to adsorb and reach an equilibrium. Without adsorption the generation of hydroxyl radicals was more difficult and degradation was lower. This effect is visible for the experiment at pH=8 where degradation was very low (see results shown in Table 4.1).

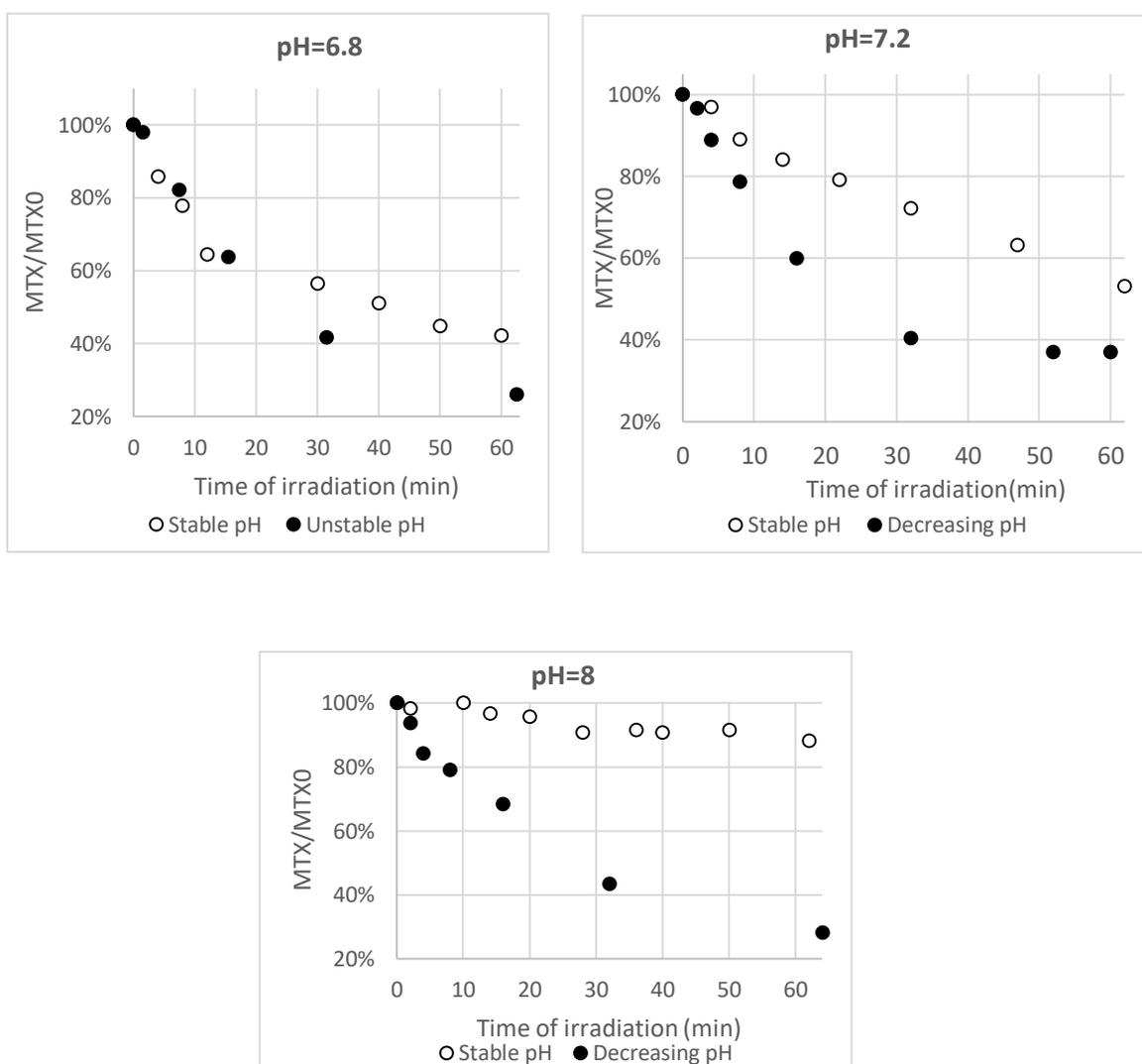


Figure 4.9: Effect of stable and decreasing pH.
[MTX]=3.3 mg/L, [TiO₂]=0.5 g/L, 1 LP lamp (10 W, 254 nm)

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4.2.5 Effect of catalyst load

Some experiments were performed with different photocatalyst load (0.25 g/L; 0.5 g/L; 1 g/L; 1.5 g/L and 2.5 g/L) to understand the effect of the photocatalyst load on the kinetic of degradation. It was decided to work at pH=8 to completely isolate the effect of adsorption because it proved to prevent degradation. In fact, at pH=8 estimated adsorption is zero.

Figure 4.10 shows the results obtained at different photocatalyst loads.

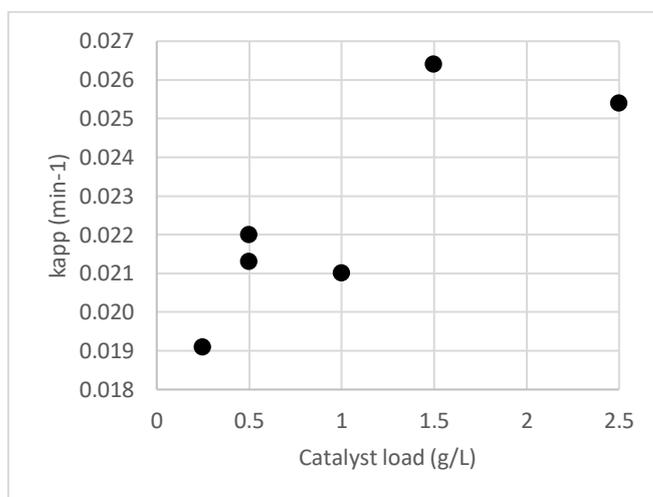


Figure 4.10: Effect of photocatalyst load.
[MTX]=3.3 mg/L; initial pH=8; 1 LP lamp (10 W, 254 nm)

The efficiency was quite similar for all the photocatalyst loads tested but k_{app} seems to reach a plateau at 1.5 g/L. This is a typical behaviour in photocatalytic applications since over a certain value of photocatalyst load the solution becomes too turbid and TiO₂ tends to form bigger agglomerates. An increase in turbidity prevents light to penetrate deeply in the medium making TiO₂ particles far from the light source more likely to be activated. Instead, an increase in the size of the particles corresponds in a decrease of their specific surface area and in the total area exposed to irradiation.

It is also important to define the optimum value of photocatalyst load to avoid wasting the reagent. Other studies about the photocatalytic oxidation with of cytotoxic compounds with Degussa P25 assessed the optimum value for the photocatalyst load. The results are summarized in Table 4.2.

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Table 4.2: Optimum photocatalyst load for Degussa P25 in other studies

Compound	Concentration (mg/L)	Photocatalyst load range (mg/L)	Optimal photocatalyst load (mg/L)	Ref.
MTX	20	100-1000	500	[1]
MTX	15	-	200	[2]
5-FU	0.02-0.2	5-100	20	[3]
5-FU, CF	0.2	-	20	[4]
IF	50	1250-5000	5000	[5]

[1] Lutterbeck *et al.*, 2015; [2] Calza *et al.*, 2014; [3] Lin, Lin, & Hung, 2014;

[4] Lin *et al.*, 2014; [5] Ofiarska *et al.*, 2016

There is not a general value for the optimum photocatalyst load since this value is a function of the concentration of the compound to be oxidised as a higher amount of contaminant needs a higher amount of radicals for its degradation. It is necessary to increase the quantity of TiO₂ to have more active sites for OH[•] radicals formation (Neppolian *et al.*, 2002).

In a review on the photocatalysis of dyes (Konstantinou & Albanis, 2004) it is reported that degradation does not enhance over 2000 mg/L as observed by us. Herrmann reported this limit to be 2500 mg/L (Herrmann, 1999).

The optimum photocatalyst load depends also on the fluid dynamics inside the reactor that impacts on particle aggregation. Also lamp power influences light penetration in the medium and so the optimum value for TiO₂ dosage.

4.2.6 Effect of initial MTX concentration

MTX concentration varied from 1 to 5 mg/L. No experiments were performed at an initial concentration under 1 mg/L as the concentration of MTX reached the LOD of the spectrophotometer during degradation. All the experiments were performed at [TiO₂]=0.5 g/L and NaOH was dosed to adjust the pH at 8.5 before starting irradiation. This pH value was chosen to ensure no adsorption of MTX took place. The results are reported in Table 4.1 and Figure 4.11.

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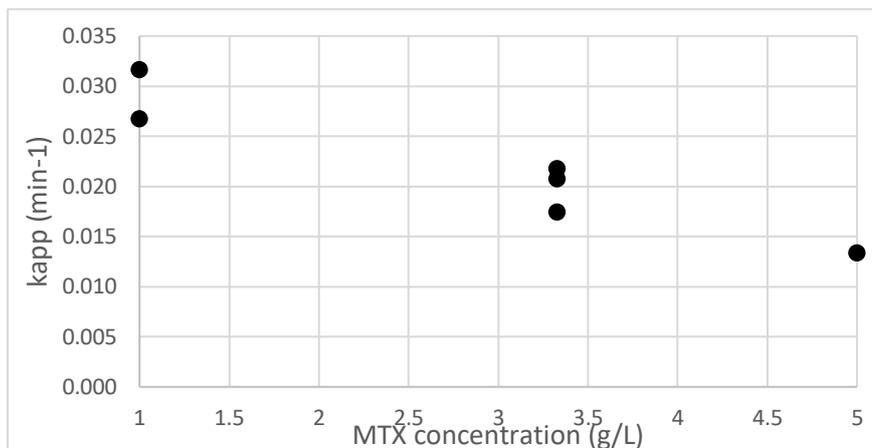


Figure 4.11: Effect of MTX concentration

[TiO₂]=0.5 g/L; pH=8.5; 1 LP UV lamp (10 W, 254 nm)

Results underline a dependency between the initial concentration and the k_{app} as if the concentration decreases, k_{app} increases. Other authors who worked with antineoplastic compounds described a similar behaviour (Table 4.3).

Table 4.3: Dependency between concentration and k_{app}. Results from other studies.

Compound	Photocatalyst	Concentration range (µg/L)	Optimal concentration (µg/L)	Ref.
IF	Degussa P25	100-2000	100 k _{app} ↓ with C ↑	[1]
IF	Pt-doped-TiO ₂	5000-50 000	5000 k _{app} ↓ with C ↑	[2]
IF	Bi-B-TiO ₂	5000-50 000	5000 k _{app} ↓ with C ↑	[3]
5-FU, CP	Degussa P25	20-200	200 k _{app} ↑ with C ↑	[4]

[1] Lai et al., 2015; [2] Ofiarska et al., 2016; [3] Fiszka Borzyszkowska et al., 2016;

[4] Lin & Lin, 2014

Photocatalysis is faster for decreasing MTX concentrations probably because an higher amount of cytotoxic drug in solution can absorb more photons preventing their absorption by TiO₂. In addition, a too high amount of the compound to degrade can saturate the photocatalyst surface and de-activate it.

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Similar results have been obtained in the photodegradation of other contaminants (Gad-Allah *et al.*, 2011; Ochuma *et al.*, 2007; Toor *et al.*, 2006).

However, the experiments of Lin and Lin (Lin & Lin, 2014) showed an opposite trend with the k_{app} increasing with concentration and reaching a plateau. This was because the authors worked at much lower concentrations than the ones tested in this study (two orders of magnitude) and at those very low concentrations the probability of reaction between the compound and the radical species in solution is lower.

4.2.7 Optimized conditions for degradation

The assessed optimal conditions for the photocatalysis of MTX were:

$$[\text{MTX}] = 1 \text{ mg/L}; \quad [\text{TiO}_2] = 1.5 \text{ g/L}; \quad \text{Initial pH}=7;$$

Experiments at these conditions lead to the highest values of k_{app} , respectively **0.0608 min⁻¹** ($R^2=0.9727$) and **0.0663 min⁻¹** ($R^2=0.9358$). These values are more than twofold higher than the ones obtained for other experimental conditions (see Table 4.1: Summary of the results (1 LP UV lamp, 60 min of irradiation)).

4.2.8 Effect of the lamp

In order to understand how the light source can influence photocatalysis some experiments were performed changing the lamp.

A medium pressure UV lamp was tested and another experiment was carried out placing two low pressure germicide UV lamp in the reaction vessel.

As low and medium pressure lamps differ both for the lamp power and for the wavelength of irradiation, the k_{app} calculated for each experiment was normalized for the lamp power and for the volume of the irradiated medium. In fact, it was necessary to remove the top part of the reaction vessel to place two lamps, implying to work with 1 L of solution to avoid spilling water outside.

The procedure of normalizing the k_{app} for the value of the light intensity and the volume is an approximate way to compare the performance of different lamps only in terms of the irradiation wavelength. Leblebici *et al.* used a similar method to compare different photocatalytic set-ups (Leblebici, Stefanidis, & Van Gerven, 2015). It is necessary to isolate the effect of the light intensity as the more powerful is the lamp the higher is the quantity of photons released to activate TiO₂.

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According to Mozia (Mozia, 2010) at low light intensity (0-20 mW/cm²) the dependency between light intensity and reaction rate is linear.

The formula applied to normalize the value of k_{app} is described by Eq. 4.1.

$$k_{app, std}(\text{min}^{-1}/W) = \frac{k_{app}(\text{min}^{-1})}{LP (W)} = \frac{k_{app}(\text{min}^{-1})}{P (W)} \cdot \frac{V (L)}{1 L} \quad \text{Eq. 4.1}$$

where:

- $P (W)$ = lamp power;
- $LP (W)$ = standardized lamp power = $P (W) \cdot \frac{1L}{V(L)}$;
- $V (L)$ = volume of the medium in the reactor;

Results are summarized in Table 4.4.

Table 4.4: Effect of the lamp [MTX]=3.3 mg/L; [TiO₂]=0.5 g/L; initial pH=7

Lamp	Lamp power (W)	λ (nm)	V (L)	k_{app} (min ⁻¹)	R ²	$k_{app, std}$ (min ⁻¹ ×W ⁻¹)
1 LP	10	$\lambda_{max} = 254$	1.5	0.0280	0.9982	0.00420
2 LP	10 + 10	$\lambda_{max} = 254$	1	0.0761	0.8305	0.00381
1 MP	125	200-400	1.5	0.1034	0.7398	0.00124

As expected, degradation was faster in the case of the low pressure lamp because, compared to the medium pressure lamp, its spectrum is narrower and concentrated around 254 nm.

However, commercial TiO₂ has an energy band gap of 3,2 eV and can be activated by light having $\lambda < 390$ nm. This explains the photodegradation observed with the medium pressure lamp (see spectrum in Figure 3.4).

Regarding the addition of a second low pressure lamp, results shows that no enhancement of degradation occurred. This can be due to the reciprocal position of the two lamps in the reactor as they were placed along the central diameter of the cylindrical vessel and one lamp screened the radiation of the other.

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4.2.9 Effect of the matrix

One experiment was carried out in synthetic urine in order to assess if it is possible to photo-degrade MTX in a more complex matrix.

Results showed that in the presence of synthetic urine the photo-oxidation was inhibited (see Figure 4.12). Two factors can interfere with the photocatalytic process: the system pH and the presence of other compounds that can inhibit degradation.

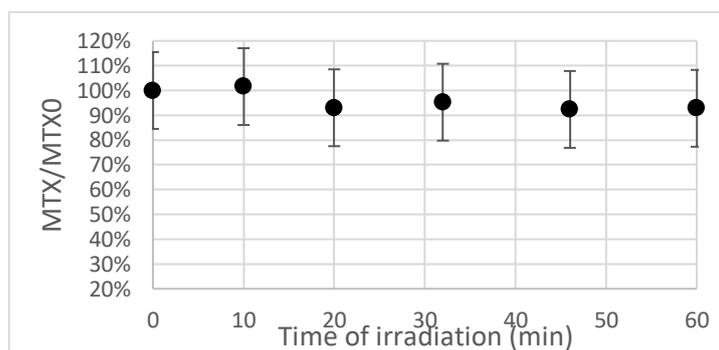


Figure 4.12: Degradation in synthetic urine
[MTX]=10 mg/L, [TiO₂]=1.5 g/L, pH=5.6, 1 LP lamp (10 W, 254 nm)

The pH of the urine was 5.6 and experiments on adsorption showed that at acidic pH an important amount of the spiked MTX can adsorb on the TiO₂ preventing the degradation since no active sites are free for the production of radicals.

The presence of other substances in the matrix can slow down or block the process as other ions can adsorb on the photocatalyst surfaces occupying active sites or/and scavenge hydroxyl radicals.

Adsorption on the photocatalyst lead at the same consequences shown by MTX (see sections 4.2.1 and 4.2.4) as the radical scavenging can lead to a slow kinetic because there is competition in radical consumption by the target compound and other ions. In particular, anions oxidize to the corresponding radical that can react with MTX; however, typically these radicals have less oxidising power than hydroxyl radicals and oxidation is slower compared to hydroxyl radical oxidation.

The presence of ions as Cl⁻, H₂PO₄⁻/HPO₄²⁻/PO₄³⁻, HCO₃⁻/CO₃²⁻ and HSO₄⁻/SO₄²⁻ deriving from the dissociation of the compound in solution (see Table 3.2) can modify the degradation mechanism.

Some authors underline that, as Cl⁻ and SO₄²⁻ have no impact on the kinetic of degradation and phosphate ions can enhance it, HCO₃⁻/CO₃²⁻ can strongly inhibit the

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process (Zhu, Nanny, & Butler, 2007). Other studies proved the negligible role of chloride (Choi *et al.*, 2014) and the positive action of phosphate ions (Park, Lee, & Kim, 2017).

On the opposite alkalinity is a strong scavenger as reported by many authors (among them: Autin *et al.*, 2013; Pelaez *et al.*, 2011 ; He *et al.*, 2012). According to Autin *et al.*, alkalinity inhibits UV/TiO₂ photocatalysis as it scavenges radicals and leads to an increase in particles size (less surface area and less photon absorption) (Autin *et al.*, 2013).

Also the presence of DOC (in the form of creatine, urea, acid and ascorbic acid) decreases the removal efficiency as it can both scavenge radicals and saturate the surface of TiO₂. Some authors found that the degradation efficiency decreases with an increase in DOC (Autin *et al.*, 2013; Choi *et al.*, 2014). In fact, Lai *et al.*, in another study about cytotoxic compounds cyclophosphamide and ifophosphamide, observed that photocatalysis in real pharmaceutical effluents was blocked due to the high content of DOC (Lai *et al.*, 2015).

4.3 Photocatalysis of the mix of contaminants

After having optimized the procedure for MTX, the photo-catalytic set-up was tested on the mixture of the three cytotoxic compounds together (MTX, GEM, CP). Two experiments to assess adsorption of GEM and CP at different pH and photolysis were performed before try to degrade the compounds at different initial pH values.

4.3.1 Adsorption of the mix of contaminants

The adsorption of the contaminants as function of the pH was assessed. The concentration of the three compounds was measured by HPLC.

Results are represented in Figure 4.13.

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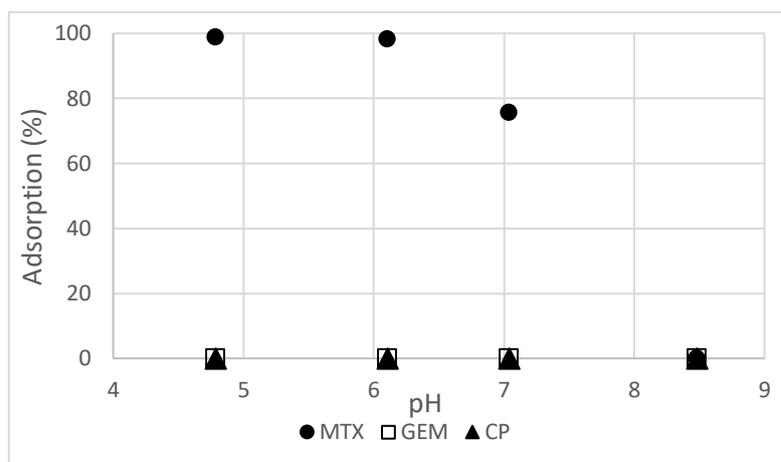


Figure 4.13: Adsorption of MTX, GEM, CP measured by HPLC.
[MTX]=500 µg/L; [CP]=100 µg/L; [GEM]= 100 µg/L; [TiO₂]=0.5 g/L,
time of adsorption = 30 min

Among the three compounds only MTX showed adsorption (see also section 4.2.1). Other authors obtained same results for CP with pure TiO₂ (Lin & Lin, 2014; Lin, Lin, & Hung, 2014a; Ofiarska et al., 2016). To our knowledge, no study about GEM adsorption on TiO₂ has been done yet.

Differences among the adsorption of the three compounds are probably due to their different chemical structure.

4.3.2 Photolysis of the mix of contaminants

A quick preliminary experiment was performed to assess the photolysis of the mix of all the target compounds. The mixture was irradiated for 20 minutes.

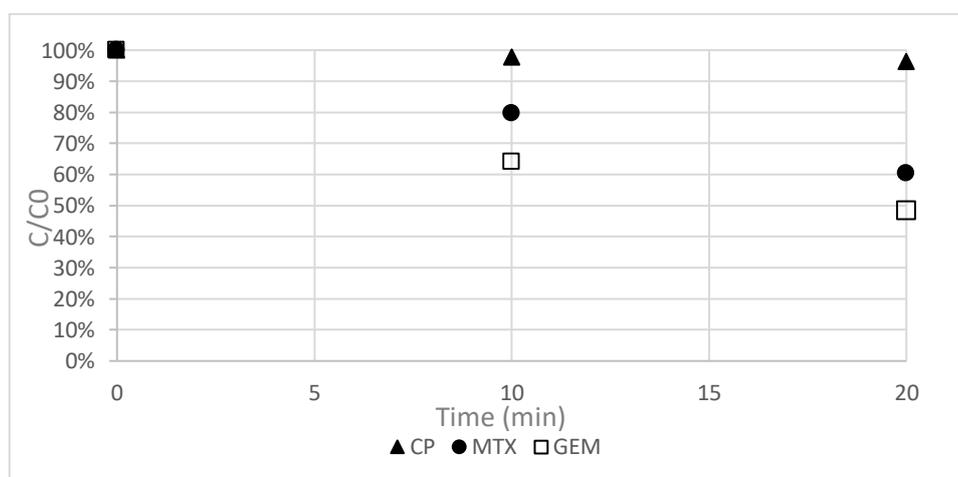


Figure 4.14: Photolysis of the mix of contaminants.
[MTX]=100 µg/L, [CP]=100 µg/L; [GEM]= 100 µg/L, pH=4.8, 1 LP lamp (10 W, 254 nm)

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Results in Figure 4.14 show that among the three contaminants only CP does not degrade by photolysis while MTX and GEM show degradation without photocatalyst.

4.3.3 Effect of pH on the photocatalysis of the mix of contaminants

The effect of three different pH values on the degradation of the mixture of the three compounds was studied. The three pH values were:

- pH=5,8 (optimal pH for CP degradation according to *Lin & Lin, 2014*),
- pH=7 (optimal pH found for MTX photocatalysis),
- pH=8 (negligible MTX adsorption).

No optimal pH for GEM was tested as to our knowledge no study about the photocatalysis of this compound with TiO₂ has been conducted yet.

All the results are summarized in Figure 4.15 and Table 4.5.

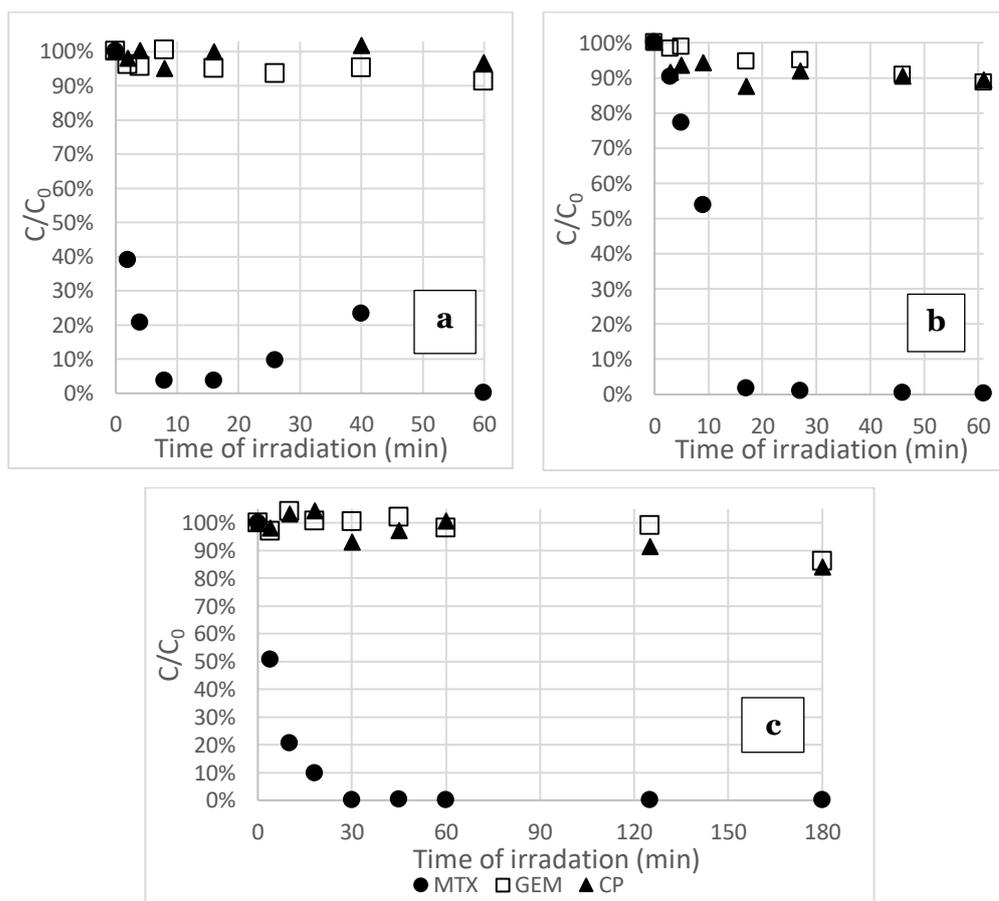


Figure 4.15: Effect of initial pH on the mix of contaminants: pH=5.8(a), pH=8 (b), pH=7 (c).
 [MTX]=100 µg/L, [CP] =100 µg/L; [GEM]= 100 µg/L; [TiO₂]= 1.5 g/L; LP UV lamp (10 W, 254 nm)

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The increase in concentration after 20 minutes is probably due to a desorption of the adsorbed MTX as adsorption was very high at pH=5.8 (97.8%). However, the difference in concentration between the point at 8 min (132 ng/L) and the point at 40 min (854 ng/L) is 722 ng/L and can be linked to heterogeneous stirring conditions in the reaction vessel.

Table 4.5: Degradation and adsorption of MTX, GEM and CP

		Concentration (µg/L)	Ads (%)	η _{deg} (%)
pH=5.8 T _{irr} =60 min	MTX	100	97.8	100 ^(*)
	CP	100	0	2.6
	GEM	100	0	7.9
pH=7 T _{irr} =180 min	MTX	100	22.7	100 ^(*)
	CP	100	0	17.6
	GEM	100	0	16.2
pH=8 T _{irr} =60 min	MTX	100	2.4	99.8 ^(*)
	CP	100	0	6.6
	GEM	100	0	9.6

^(*) η_{deg} of MTX refers only to the parent compound

Results showed that the optimized procedure for MTX was not effective on the two other contaminants.

More in detail, degradation at pH=5.8 was probably due to only photolysis since the photocatalyst surface was completely covered by MTX. In this case, the presence of TiO₂ was deleterious to degradation as photolysis of GEM was inhibited by the increased turbidity of the solution.

At the other two pH values MTX was readily decomposed as GEM and CP were more recalcitrant. A cause to this phenomenon could be the higher scavenging effect of MTX in comparison to GEM and CP.

Best results were obtained at pH=7 that is close to pH_{PZC}. at which the photo-catalyst exhibits neutral charge and no repulsion establishes with the cytotoxic compounds. In fact, all these compounds have pKa lower than pH=8 (Table 2.2) and at pH<8 exhibit negative charge as the catalyst (pH>pH_{PZC}). This is a less favourable situation than pH=7 as the photocatalyst and the compounds repel.

5 CONCLUSIONS

With the present work degradation of MTX by TiO₂-UV photocatalysis was demonstrated. The procedure was optimized in term of pH, catalyst load and MTX concentration. At the optimal conditions (initial pH=7, [TiO₂]=1.5 g/L, [MTX]=1 mg/L) MTX was degraded in less than 10 minutes by irradiation with a low pressure UV lamp (10 W, 254 nm) in pure water. The rate of the process increased for increasing TiO₂ load and decreasing MTX concentration. pH showed to be an important parameter regulating adsorption on TiO₂ and the rate of the degradation. At acidic pH MTX was completely adsorbed and the photodegradation was inhibited probably due to excessive TiO₂ surface coverage; best k_{app} was obtained for pH=7 that is close to the PZC of TiO₂ as at higher pH the negative charge of TiO₂ repelled deprotonated MTX molecules (pK_a=4.8). One experiment was performed spiking MTX in synthetic urine showing that a complex matrix may hinder the degradation probably due to the presence of radical scavengers as inorganic ions and NOM.

At the end the procedure was tested on a mixture of MTX, GEM and CP at different initial pH. As MTX was completely degraded GEM and CP showed partial degradation after 180 min of irradiation ($\eta_{GEM}=16.2\%$ and $\eta_{CP}=17.2\%$) probably because MTX and its TPs are more affine to radical attack. So, the treatment seemed to be effective towards cytotoxic compounds depending on their chemical structure but further studies are needed.

Future research will be addressed to test the technique on a pilot-scale with real HWWs in order to assess the efficacy of the process on broad range of pharmaceuticals. Eco-toxicity tests will be performed to understand if the process generates toxic TPs.

The photocatalytic setup will be combined with a membrane to recover TiO₂ particles and Cu-doped photocatalyst will be used to test if sunlight can activate photocatalysis.

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APPENDIX A

SAMPLING PROCEDURE

The sampling procedure followed these steps:

- 1) Extract 8 mL aliquot of solution with a 25 mL syringe connected to a Teflon tube;
- 2) Connect the cellulose filter to the syringe;
- 3) Filter first 6 mL through the 0.2 μm filter and discharge them into the waste container;
- 4) Filter the last 2 mL into a glass vial to be sent to analysis;
- 5) Clean the cellulose filter with 20 mL of pure water before filter a new sample to avoid contamination from the previous one; change the filter if too high pressure is needed to filter pure water;
- 6) Clean the syringe and the Teflon tube connected to it two times extracting 20 mL of demineralized water and discharge it into the waste container. Clean the syringe with other 20 mL of pure water;
- 7) Dry the Teflon tube pumping air from a clean syringe ensuring that no water residues remained in it to avoid dilution of the next sample;
- 8) Repeat the procedure starting from point 1);

APPENDIX B

FUNDAMENTALS OF HPLC

HPLC/MS is a technique to separate compounds in a mixture even if they are at very low concentrations (ng- μ g/L).

The process is articulated in two phases: chromatography (HPLC) and mass spectrophotometry (MS).

HPLC exploits the differences in affinity between each compound contained in a liquid solution (mobile phase) and a solid phase (stationary phase). The liquid mixture containing the analytes is forced to pass through a column of adsorbent material, the stationary phase, thanks to high pressure pumps. The stationary phase is typically made of a granular material such as silica packed in a column and working pressures can reach hundreds of bars. Regarding the mobile phase, it is generally a mixture of solvents such as water, acetonitrile or other organic substances.

As every different compound has a different degree of interaction with the stationary and the mobile phase, analytes in solution are eluted through the column at different flow rates. This difference in flow rate and so in the elution time, the so-called *retention time*, allows the separation and detection of each compound. To reduce the time to separate the compounds pumps can mix different solvents together in different ratios during time generating a composition gradient in the mobile phase.

As the process is based on adsorption principles, the temperature plays an important role in the interactions between the mobile and stationary phases and has to be kept constant during all the process.

A detector placed after the elution column allows the measure of the concentration of different compounds characterized by their own retention times. Typically, the

APPENDIX B

detector measures UV absorption to correlate the amount of the absorbed radiation to the concentration of the compounds. Calibration of the instrument allows to establish a relationship between absorption at a specific wavelength and the concentration of a target compound.

If HPLC allows the separation of the compounds, mass spectrometry helps to recognize them. The mass spectrophotometer works ionizing the compounds and subsequently fragment and separate them in function of their mass-to-charge ratio (m/z). Mass-to-charge ratio (m/z) is the ratio between the molecular mass number (m) and the charge number of the ion (z).

First of all, a ionized stream of ions is created and then electro-magnetic forces separate the charged particles according to different m/z ratios. In fact, the dynamics of the ions can be described combining Newton's 2nd law of motion (Eq. B.) and the expression of the Lorentz force (Eq.B.2).

$$\mathbf{F} = m\mathbf{a} \tag{Eq. B.1}$$

$$\mathbf{F} = Q(\mathbf{E} + \mathbf{v} \times \mathbf{B}) = ze(\mathbf{E} + \mathbf{v} \times \mathbf{B}) \tag{Eq. B.2}$$

The combined equation (Eq. B.3) describes the motion of the ions in function of their m/z .

$$(m/z) \cdot (1/e) \cdot \mathbf{a} = (m/z) \cdot (1/e) \cdot \frac{d^2s(x, y, z)}{dt^2} = \mathbf{E} + \mathbf{v} \times \mathbf{B} \tag{Eq. B.3}$$

The meaning of the variables in Eq. B.1, B.2 and B.3 is the following:

- m = mass of the ion;
- z = charge number of the ion;
- e = elementary charge of an electron= $1,609 \times 10^{-19}$ C;
- \mathbf{a} = acceleration;
- $s(x,y,z)$ = position of the ion in the (x, y, z) space;
- \mathbf{E} = electric field;
- \mathbf{v} = velocity of the ion;
- \mathbf{B} = magnetic field;

So according to Eq. B.3 ions in the stream change their trajectories depending on their m/z . Varying the intensity of the electro-magnetic forces allows the transmissions of ions with a m/z belonging to a target range to reach a final detector.

Mass spectrophotometry is useful also to recognize the structure of a compound as the compound is progressively fragmented in the process and each fragment is characterized by its own m/z ratio.

Mass spectrophotometry works in three steps: ionization, mass filtration and detection.

The aim of ionization step is to create a stream of charged ions to be subsequently separated during the mass filtration step.

The apparatus used in this study charges the compound with Electro Spray Ionization (ESI) in positive mode, i.e. it creates positive particles. ESI nebulizes the mixture containing the compounds thanks to electricity. The liquid mixture is injected in a chamber through a capillary subject to a high voltage; thanks to this high voltage the meniscus at the apex of the capillary deforms into a cone (*Taylor cone*) that emits a jet stream of droplets due to repulsion between charged particles. Droplets become more and more charged till reaching the maximum amount of charged particles they can carry and dissociate creating a stream of charged ions. The apparatus decreases the droplets size thanks to evaporation caused by the injection of heated nitrogen gas ($T=350^{\circ}\text{C}$) next to the injection nozzle. The sheath gas aims to confine the ions in a precise region of the electro spray leading them to the following step of mass filtration. A circular arrangement of six capillaries (*hexabore capillary sampling array*) allows the jet stream of ions to reach the following analytical steps minimizing the fraction of ions that disperse.

The mass filtration step aims to eliminate neutral species and atmospheric air from the ion flow to reduce the noise of the measure. First of all, the atmospheric air is eliminated by two consecutive ion funnels where the ions are drawn by high voltage applied. The first funnel is at high pressure as the second funnel operates at low pressure. The first funnel is offset from the capillary arrangement and the inlet of the second funnel to facilitate the remove of neutral species not subject to electrical forces.

APPENDIX B

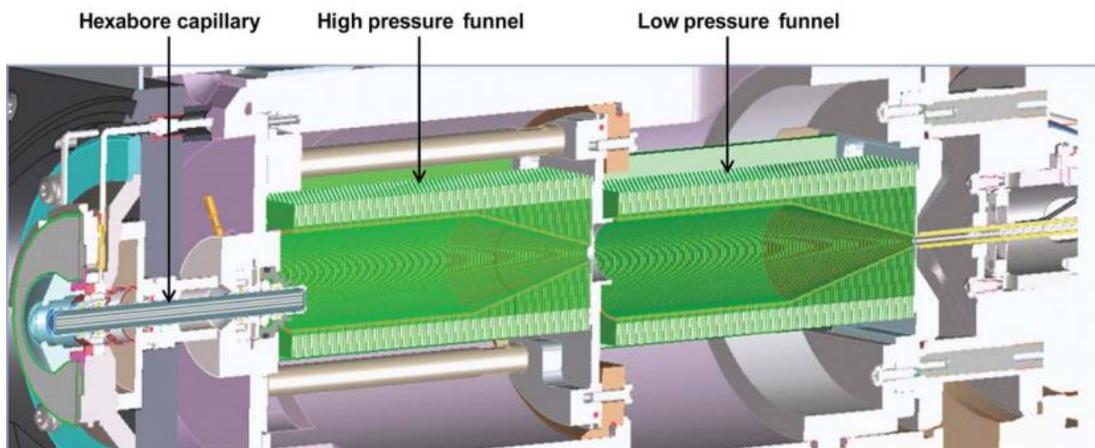


Figure B.1: Schematic representation of the dual ion funnel

(http://www.agilent.com/cs/library/technicaloverviews/Public/5990-5891en_1o%20CMS.pdf)

Ions exiting from the low pressure funnel enter an *octopole ion guide*, i.e. an arrangement of 8 tubular rods that maintain a high voltage, that drive only ions of the targeted masses to the first quadrupole mass filter. Ion guides allows the transmission of low kinetics energy ions without substantial losses. A quadrupole mass filter exploits a changing electric field to confine ions motion in a specific direction.

A hexapole collision cell (second quadrupole) follows the first quadrupole to further improve separation before the last step of mass filtration in the third quadrupole.

For the complexity of the functioning of quadrupoles see *Prof & Fiehn, 2008*.

The last step of mass spectrometry is the detection step where the selected ions impact a high voltage conversion dynode that functions as an electron multiplier to increase the signal arriving at the detector.