Photodegradation of Drugs in Wastewater, Comparative Correlated Recalcitrance Analysis and Related Problems

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Introduction

The World Health Organization defines health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity".

Therefore the pharmaceutical production of the last 10 years has been not only addressed to cure the illnesses as existing, but also to contribute to the reaching of this wealth.

All that, however, has risked and still it risks, to cause an unjustified drug abuse with deleterious consequences both on the health itself of the man, and on the delicate ecosystems; in fact, the drugs reach streams and rivers spreading easily to the environment.

The presence of such substances negatively affects the ecosystem and, due to bioaccumulation, it is possible their transfer into the food chain.

Even though the presence of drugs in waters has been already investigated for several years do not exist sufficient data regarding their recalcitrance to the natural degradation. A comparative recalcitrance study is necessary both to analyse the true environmental persistence and evaluate new and old methods of artificial molecular degradation.

The public opinion has become well aware, because of heavy accidents, of the diffusion and riskness of other substance categories as, for instance, pesticides, herbicides, parasiticides and, even if at small concentration, the industrial dyes.

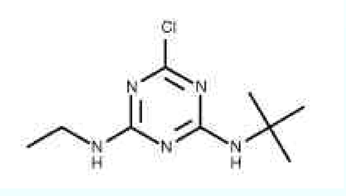

Fig. 1, Our representative herbicide, Terbuthylazin, CAS: 5915-41-3, a herbicide used to control a wide range of weeds and as pre-emergence herbicide

For the synthesis of these organic molecule classes, are used techniques of drug design fitted to make them resistant to photolysis, bacterial and chemical degradation typical in the environment.

Two of these molecule classes, the industrial dyes and pesticides-herbicides, are "selected" and/or "planned" to give persistence in environment, to resist for long periods in storage, transport and marketing so that they remain unchanged till the end of their use. They must be more resistant to the action of the solar radiation and, for pesticides, the persistence plays a fundamental role both in the utilisation and in the environmental pollution.

For a lot of drugs there are also studies about accelerated ageing [1] and through ours and other similar works we intend to build a recalcitrance scale comparing these molecule classes.

Several European projects were dedicated to the study of persistence of organic molecules in the environment and one in particular to the drugs in waters. In the context of one of them (Rempharmawater) were investigated the incoming and the outcoming waters of a Sewage Treatment Plants (STP) of Latina a small town of south Lazio discovering little but significant drug concentrations.

As result, a scale of recalcitrance of 4 drugs was produced in comparison with a pesticide and a dye.

In Fig. 1 the structural formula of Terbuthylazin is shown. The total concentration of Terbuthylazin in treated forest soils decreased by a factor of approximately 2 over the course of one growing season. The half-lives for degradation (mainly soil catalysed hydrolysis) of Terbuthylazin in Hatzenbuhl soil at pH 4.8 and Neuhofen soil at pH 6.5 are 84 and 170 days, respectively (see: http://www.speclab.com/compound/c5915413.htm).

Fig. 2, Our representative dye, Acid Blue 29, CAS: 5850-35-1, a dyes widely used in textile, leather, paper colouring, ink jet cartridges, etc.

The structural formula of Acid Blue 29 is shown in Fig. 2. This dye is widely used in textile industry, for colouring hides, to fill the cartridges of the printers' inkjet and in other industrial sectors. It is known for its resistance to the decoloration, but at low concentrations it is difficult to notice with naked eye as the resulting colour is very weak.

European Projects

Before studying an effective abatement method is necessary to investigate, or at least to assume, how the pharmaceutical active principles reach the waters.

Drugs have several ways to reach the superficial waters:
- by incomplete metabolisation of an organism with the consequent excretion of the starting products and metabolised compounds;
- by uncontrolled draining away, also involuntary, both by the patients and by producers or distributors still unconscious of the problem;
- by incorrect use (fraudulent, doping, wrong proportions) by people and by animal breeding in farms.

It would be necessary add to these the draining with the same ways of veterinary drugs, disinfectant, antibacterial and plant protection products which are now heavily used in every field. Perhaps, some of them may be assimilated to drugs (having molecules with similar structures) and are produced in huge amounts and used almost without control.

So arose the problem about the presence of pharmaceuticals in planet waters (see Fig. 3, "Drug found in stirs concern fish area" by Scott Streater, Star-Telegram, at http://www.dfw.com/mld/startelegram/news/state/7036372.htm?1c) and in the lower organisms. These molecules are transported into the food chain and also in superior organisms.

Texas, il Prozac fa "felici" i pesci di un laghetto

NEW YORK - Pesci felici in un lago del Texas: il tossicologo Bryan Brooks della *Baylor University* ha scoperto che la fauna ittica del lago di Lewisville, vicino a Dallas, ha involontariamente consumato il potente anti-depressivo Prozac. Tracce di fluoxetina (che ha effetti rilassanti), il composto attivo della "pillola della felicità", sono state trovate nelle branchie dei pesciolini. Sarebbero arrivate nelle acque blu del laghetto dagli scarichi di un depuratore inadeguato a filtrarle. E dai pesci felici del Lewsville Lake, il Prozac avrebbe fatto ritorno, sotto mentite spoglie, nei pasti degli abitanti della contea di Denton, molti dei quali sono accaniti pescatori. "La mia è una preoccupazione da ambientalista: dobbiamo capire perché una sostanza chimica scaricata nell'ambiente sia potuta arrivare a inquinare il lago". (ANSA)

Fig. 3, © Reuters Limited, Dallas, Oct. 23 2003 .

The importance of such problems is also highlighted by several initiatives of EU with the purpose to monitor the presence of drugs and other pollutant molecule in the environment and to look for methods for their correct and effective abatement.

a) ERAPharm (Environmental Risk Assessment of Pharmaceuticals): the objective of ERAPharm is to improve and complement existing knowledge and procedures for the environmental risk assessment from human and veterinary pharmaceuticals. It investigated unstudied major routes leading to exposure of the terrestrial and aquatic environment, the fate of pharmaceuticals in surface water and sediment and the effects of antibiotics on microbial communities to the spread of genetically encoded resistance. Duration 36 months;

b) AquaStress (Mitigation of Water Stress Through new Approaches to Integrating Management): the AquaStress project generated scientific innovations to improve the understanding of water stress and the development of supporting methods and tools to evaluate different mitigation options and their potential interactions with environment. Duration 48 months;

c) Rempharmawater (Ecotoxicological Assessments and Removal Technologies for Pharmaceuticals in Wastewater): the present project aims at the prevention of pollution of surface-water resources and more generally at the protection of the environment. Therefore the ultimate aim is represented by the achievement of results which can improve living conditions in Europe through the minimisation of environmental impact of wastes posing serious risks to human health. The project focuses on "Ecotoxicological assessments and removal technologies for pharmaceuticals in wastewater", also focus on database development, *Pharmafic*, to store data (ecotoxicological, physical and chemical properties, etc) on the drugs studied. Duration 36 months;

d) Eravmis (Environmental Risk Assessment of Veterinary Medicines in Slurry): this report investigates the possibility of defining scenarios for exposure and distribution models for the environmental risk assessment of veterinary medicinal products at registration. A critical component of any modelling procedure is the identification of relevant scenarios to characterise the environmental conditions determining model input parameters. The study is extended to residues of veterinary medicinal products, which reach the environment through spreading of slurry on agricultural soil and animal husbandry. Duration 36 months;

e) Poseidon: the acronym POSEIDON represents the project "Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse". The project defines the activities of the EU in the field of research, technological development and demonstration for the period of 1998-2004. Duration 36 months.

f) WSSTP (Water Supply and Sanitation Technology Platform): it is a European initiative, open to all stakeholders involved in European water supply and sanitation and major end-user groups. The participants in the platform will together produce a common vision document for the whole European water industry together with a strategic research agenda and an implementation plan for the short (2010), medium (2020) and long term (2030).

drugs	sample1	sample2	sample3
Gemfibrozil	0.81	0.84	4.76
Fenofibrate	0.16	0.10	0.16
Bezafibrate	n.a.	n.a.	0.91
Clofibric acid	0.68	n.a.	0.23
Ibuprofen	0.18	0.02	0.02
Flurbiprofen	n.a.	n.a.	0.34
Naproxen	0.29	0.41	5.22
Diclofenac	0.47	1.48	5.45
Phenazone	n.a.	0.37	n.a.
Acebutolol	0.04	0.02	0.11
Metoprolol	0.01	0.01	0.10
Oxprenolol	0.01	<0.01	0.03
Propranolol	0.01	0.01	0.09
Carbamazepine	0.30	0.34	0.50
Trimetoprim	0.04	0.03	0.13
Sulfamethoxazole	0.01	n.a.	0.03
Ofloxacin	0.58	0.29	0.31
Lomefloxacin	0.32	0.18	0.22
Enoxacin	0.03	0.01	0.03
Norfloxacin	0.07	0.06	0.06
Ciprofloxacin	0.07	0.06	0.04

Tab1, found concentrations, in µg/L, in STP effluents in south Lazio small city, in text methods description

Among many articles found on the subject, in which several waters are analysed measuring the drugs concentration, we signal the following one with an extensive list of active principles [2].

Following the European project for this study the following molecules were used: Terbuthylazin, Nifuroxazide, Clofibric Acid, Sulfamethoxazole, Ofloxacin, Acid Blue 29. In figures 4-7 are shown molecules, structures, Chemical Abstract Service Numbers and the principal use.

Environmental Monitoring

For their cost drugs must also have long expires to be able to reach the patient still intact and remain chemically active also after the opening of the packaging. Also all the molecules that must be kept at low temperature show a good environmental persistence.

PALMER [3] is an organisation that proposes to discipline, coordinate and develop activities aimed at the promotion, realisation and management of research programs, test experimentation and diffusion in the fields of the scientific, technological and organizational innovation, the study and the realisation of actions addressed to the environmental protection.

Agreeing with a European project, PALMER analysed the superficial waters coming from the STP of Latina.

The research was performed with two methods: liquid chromatography with electrospray detector and MS/MS, performed in SRM mode; gas chromatography with MS detector in SIM mode.

With aim of these methods were identified the active principles shown in the table 1 in the waters coming from STP at three different moments of 2003. Are shown here only the pharmaceutical principles that showed a concentration higher than the detection limit of 0.01 µg/L among the 26 molecules under test.

However, from these results it can be deduced that the active principles presence in the waters already treated by a common STP, normally well working, confirm our worries and the necessity of this work and others similar.

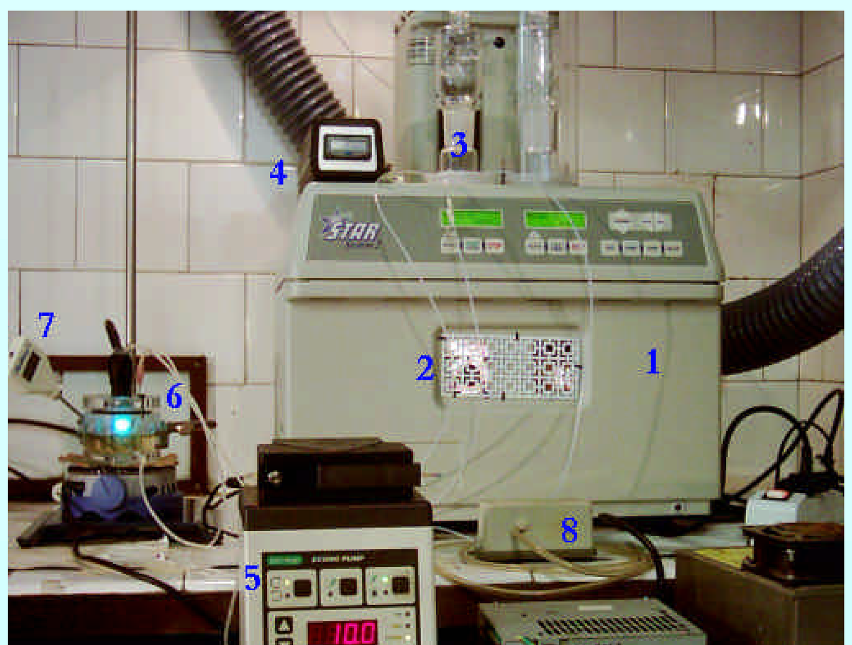

Fig. 8, Photodegradation system: 1= laboratory microwave digesting system modified by CEM Italy, both in the firmware to obtain a pulsated operation and in the hardware to obtain the window needed by the internal lamp; 2= quartz dichroic overvoltaged internal lamp, to obtain "1 sun" (with spectrum, intensity and focusing near the summer sun in the Mediterranean places); 3= cell built by the glassblower of Marbaglass in fused silica with a transmittance from 180 to 1200 nm, with volume of 50 mL and with the holes for the circulation tubing and air flows; 4= counter of magnetron impulses, useful to obtain the reproducibility of the power supply set up here for a duty cycle of 5%; 5= dual circuit peristaltic circulation pump, by means of two Teflon tubes allows circulation of solution (100 mL) in about 6 minutes and maintains the catalyst suspension; 6= above the magnetic stirrer there is the cell in PMMA, containing Hg vapors lamp mainly emitting at 254 nm, the thermometer, the stirring bar, a syringe needle for the continuos air insufflation and a hole for sampling during the degradation; 7= the digital thermometer allows to avoid the thermal microwave effect, monitoring by eyes the temperature variation (always under 40 °C), using different duty cycles; 8= membrane air pump with splitter to insufflate the filtered (0.47 µm) room air, by Teflon tubes, in the outside cell and the inside quartz cell, with the triple purpose to remove produced CO_2, to guarantee an oxygen excess in solution, to keep the catalyst in suspension; 9= double power supply to feed UV lamp in A.C. and quartz lamp in C.C. obtaining a continuos emission but with loss of life time

Spontaneous and catalysed photodegradation theory

It is necessary to define the degradation like the demolition of the molecule under investigation with the production of other molecules with smaller formula weight and, instead, the mineralisation as the complete degradation of the organic molecule with the production of CO_2, H_2O, and superior oxides of the other elements as N, S, P, etc.

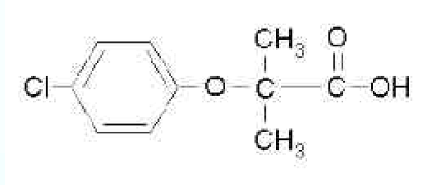

Fig. 4, Clofibric Acid, CAS: 882-09-7, it is an antihyperlipidemic that causes the decrease of plasmatic levels of cholesterol and triglycerides and increasing the activity of lipase

The Earth has a complex series of systems for the molecule degradation, we could call it *cycle of CO_2*, in which the aerobic carbon-carbon bond broken proceeds spontaneously towards the gas production.

The energy of the bond between two atoms is still very strong and it is always necessary to put in game an amount of energy at least similar to that needed to obtain its break.

In the environment the methods to obtain degradation are principally:
- photolysis: by the UV rays among the 295 up to 400 nm that reach the earth surface with values between 5 and 35 W m^2 and also cross water for few centimetres. The speed of these reactions is still very low and for this all the living organisms, in about 550 millions of years, developed methods to repair the cellular damages produced by UV;
- oxidation: the natural and synthetic organic molecules are continuously attacked by the present oxygen, in an ubiquitarian way, and in the environment they can be degraded in presence of opportune conditions;
- ozonization: for the volatile organic molecules (VOC) the mixing up with atmosphere raises them up to 15-20 Km until they meet UV rays at low wavelength and ozone (O_3) which degrade them quickly;
- photocatalysis: the degradation obtained with the help of a catalyst increases the reaction speed and allows to obtain significant yields quickly. In the environment and in water there are several natural catalysts as Fe_2O_3, Al_2O_3, and other metal oxides which accelerate very much the degradation which would spontaneously occur in very long times;
- enzymes and microorganisms, which attack the biodegradable molecules (BDOC).

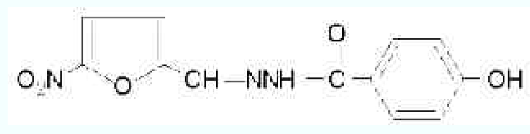

Fig. 5, Nifuroxazide, CAS: 965-52-6, it is used for the treatment of acute and chronic diarrhoea, gastro-enteritis, and colitis.
All drawing obtained from Sigma-Aldrich web site

Fig. 6, Ofloxacin, CAS: 82419-36-1, it is a synthetic antibiotic, with a wide range activity, towards aerobic and anaerobic, gram-positive and gram-negative bacteria by enzyme inactivation.

A complete list would be far and still not exhaustive.

The description, even as summary, of heterogeneous chemical-physical photolysis and photocatalysis theory, is outside the purposes of this work and many bibliographic references could be found on this subject [4, 5].

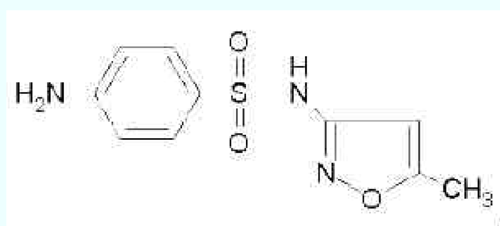

Fig. 7, Sulfamethoxazole, CAS: 723-46-6, is a sulfamide used along with trimetoprim; together they inhibit two stages in the synthesis of the acid tetrahydrofolic in bacteria and does not affect human cell.

Apparatus, Materials, Methods

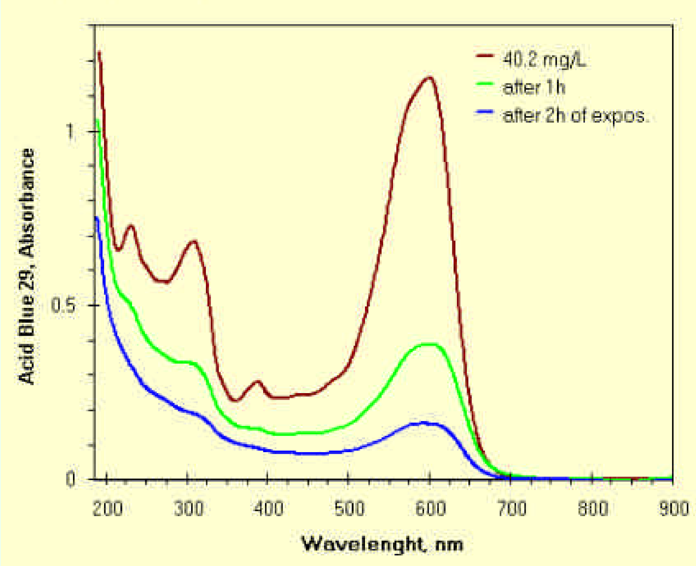

Fig. 9, Acid Blue 29, 40.2 mg/L, UV-Vis absorbance after 1 and 2 hours of exposure time in HPOP photodegradation system

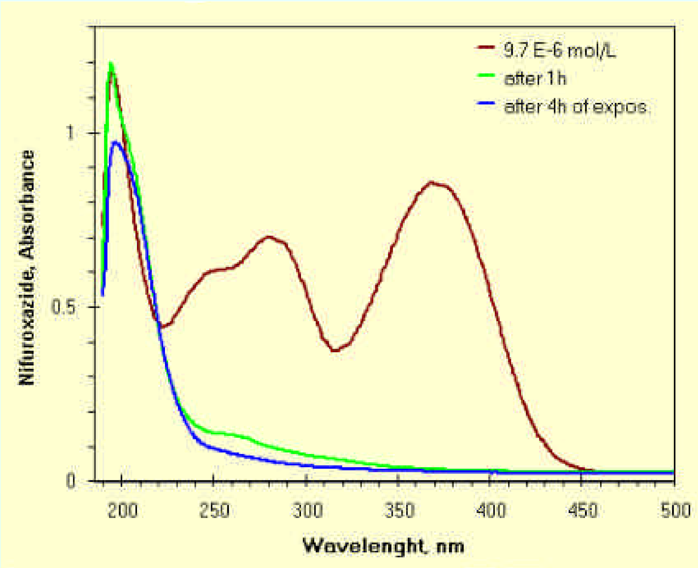

Fig. 10, Nifuroxazide, 9.7 micromol/L, UV-Vis absorbance after 1 and 4 hours of exposure time in HPOP photodegradation system

The studies on recalcitrance use catalytic photodegradation as a mean to reduce the concentration of the organic molecule [6] in order to obtain results in reasonable times.

The UV radiation and the solar radiation are already well known as efficient means for the degradation; in our case was added the radiation of microwaves which synergistically improves the efficiency and allows to attack resisting molecules in smaller time.

The historical origin of photocatalytic effect is not very well known, scientific literature start around 1930 with photocatalytic reaction of zinc oxide. In 1967-68 Akira Fujishima (a graduate student at Tokyo University), as part of their study on semiconductors, conducted research in electrophotographic imaging.

He and his professor, Honda Kenichi discovered the unique photocatalytic properties of titanium oxide. Some years later they make application of photocatalysis at water splitting and publish paper, co-authored, on the phenomenon, which subsequently came to be known as the "Honda-Fujishima effect" for heterogeneous photocatalysis [7].

Photocatalytic effect is now widely used and our research is an improvement of this study looking for efficience and inexpensive ways.

Our device (Fig. 8) is planned to use low electrical power lamps (a 3W Hg vapour UV-C and 20W halogen dichroic with about "1 sun" output) and pulsed microwaves with duty cycle of 5% with an average value of 35W, with the continuos contribution of oxygen flow in the reaction cell containing TiO_2 Degussa P25 nanomaterials as catalyst in suspension.

In these experimental conditions all the molecules are degraded obtaining such a scale of recalcitrance.

The energy balance, that is the relationship between the decrease of the concentration and the electrical power used, of this tool is positive and the synergy with the microwaves places it beyond other heterogeneous photodegradation methods.

For this technique was coined by us the acronym HPOP (High Performance Oxidation Process) for its efficiency, the favourable energy balance, the capacity to degrade every molecule.

molecule \ hours	one	two	four
Acid Blue 29	48.1	70.7	88.3
Clofibric Acid	n.a.	62.9	76.5
Nifuroxazide	68.8	n.a.	73.7
Ofloxacin	n.a.	71.6	84.2
Sulfamethoxazole	n.a.	63.4	69.3
Terbuthylazin	n.a.	1.3	10.9

Tab2, computing area under UV-Vis spectrum in a.u. before and after 1, 2, 4 hours of exposure time for the six molecules and obtaining % ratio

For all analysis were used Analytical Grade reagents, ultrapure water, and when necessary, High Purity Grade materials principally from Merck and Sigma. For the six molecules were used the pure reagents and the pharmaceutical principles.

Results

For each of the 6 molecules, it was prepared a solution of concentration 10^{-5} mol/L which is little higher than the ones discovered in the worst cases in environment and however easily detectable also after a reduction to 10%.

To study the decrease of the molecule concentration in solution it was used the UV-Vis spectrophotometry, calculating the area subtended by the absorption curve before and after photodegradation in arbitrary units (a.u) and calculating the percentages as relationship among these values. Of the three tests carried out on each molecule, the median value was used.

In Fig. 9 is shown the absorption spectrum of Acid Blue 29 and in Fig. 10 the absorption spectrum of Nifuroxazide. The decrease of the concentration of these molecules can be noticed.

In the table 2 the result of one, two and four hours decomposition is shown for the six molecules and from this table can be deduced the similitude of the 4 drugs with the dye rather than with the pesticide.

Conclusion

In this preliminary study, without reaching the complete mineralisation, substantially the recalcitrance of the studied pharmacological principles results more like that one of a dye rather than of a herbicide-pesticide using our HPOP etherogenous photocatalysis. In Fig. 11 the results obtained for the exposure time of 1, 2 and 4 hours are collected: the comparison result is even clearer.

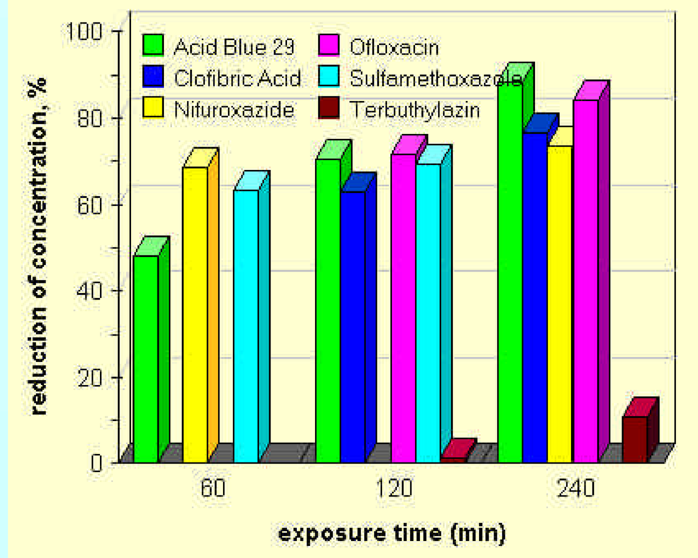

Fig. 11, Comparison of drugs with herbicide and dye. Reduction of concentration obtained by measure of UV-Vis area under spectra at different exposure times. Light sources = UV-C + 1 sun + microwave.

It is ready the project of a new device, with a lamp replacement and a modification of the system, to obtain a recalcitrance scale if using short exposure times, and the complete mineralization using longer times, opening so the possibility of an industrial application which avoids the formation of intermediate potentially more dangerous than the initial molecules.

Furthermore the study will go on with the use of commercial drugs to take also the excipient contribution into account.

We allow to wish that EU continues with these projects and launches a campaign for the "first 100 drugs (F100D)", the 100 pharmaceutical molecules (or assimilable) more discovered in the European waters.

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