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Photocatalytic degradation of cholesterol-lowering statin drugs by TiO_2-based catalyst. Kinetics, analytical studies and toxicity evaluation

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ABSTRACT

The photocatalytic degradation of simvastatin, lovastatin, and pravastatin, cholesterol-lowering statin drugs, by TiO_2-based catalyst in aqueous solutions has been studied. In all cases the degradation was found to be efficient and was clearly owing to the formation of hydroxyl radicals. When present in their open forms, the life times were evaluated, in aerated conditions, to 8.1 ± 1.2, 10.4 ± 1.3 and 19.2 ± 2.1 min for simvastatin, lovastatin and pravastatin respectively. In their lactone forms, in non-aerated conditions, the life times were found to be higher: 12.4 ± 1.1 and 15.6 ± 2.0 min for simvastatin and lovastatin. Several primary and secondary photoproducts were elucidated by means of LC–MS technique. They were obtained as a result of the addition of the hydroxyl radical to the double bonds leading to the formation of different hydroxy derivatives. Under prolonged irradiation, efficient mineralization of statin solutions was observed by means of total organic carbon (TOC) evolution. A detailed mechanism for the degradation of statins and the formation of photo-products is proposed and discussed, together with toxicity evaluation. It is stressed that photocatalysis appears to be an alternative to other chemical or biological methods for statins removal in aqueous solutions.

1. Introduction

Cholesterol lowering statins are a group of pharmaceuticals, which are the most frequently prescribed agents for reducing morbidity and mortality related to coronary heart disease. Lovastatin is a natural product; while simvastatin and pravastatin are semi-synthetic compounds [1]. Due to high persistence and widespread occurrence of lipid-regulating agents in aquatic environments, their presence in drinking water has been widely reported [1–6].

Several methods enable the analysis of lipid regulators at concentration levels of ng L^{-1} [2,5,7–11]. Quantification of statins and their human metabolites in biological matrix such as blood and urine has been extensively studied [12–21]. Similar methods development concerning statin residues in environmental samples (natural waters) present more difficulties and has not been widely investigated [1,2]. Owing to the extensive use of statins and their large scale production municipal sewage treatment plants as well as sewage treatment plants of the pharmaceutical industry might therefore be important point sources of contamination [2]. Available data for the statin class refers to the detection of atorvastatin (Lipitor) in wastewater from municipal sewage treatment plant (1–117 ng L^{-1}) and in rivers at low levels of ng L^{-1} [1,2]. Despite of common use the fate and effects of statin drugs in the environment are largely unknown.

Pharmaceuticals in general may enter the environment through different pathways, resulting in the contamination of waste or fresh water, where bacteria are most likely the primarily affected organisms. Once they are present in aqueous solutions, they can undergo photochemical transformations with sunlight via direct or indirect photoreactions [22–24]. Such photochemical degradation can be one of the major transformation processes and one of the factors that control the fate of the organic pollutants in the environment.

In this context it is useful to apply various technologies to purify aqueous municipal and industrial effluents containing pharmaceutical substances, before they enter surface waters. Among them, advanced oxidation processes (AOPs) have been the subject of major interest in recent years. These processes are characterized by the formation of the highly oxidizing species, namely hydroxyl radicals. They ensure high reactivity with low selectivity, as they are required for the degradation of different pollutants. Heterogeneous photocatalysis represents an example of AOPs capable of achieving a complete oxidation of organic and inorganic species, including pharmaceutical substances. It takes advantage of some semiconductor solids, which can be used as photocatalysts suspended in the water effluent that has to be treated, or immobilized on various types of supports. Among various solids, polycrystalline anatase

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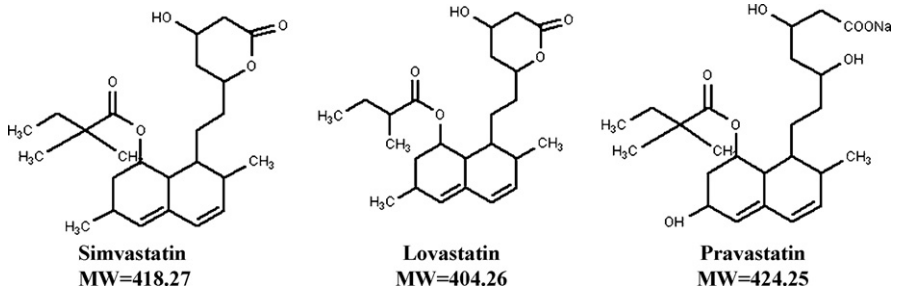


Fig. 1. Chemical structures of statins.

TiO_2 is largely used because of its low cost and its (photo)stability [25]. It is also remarkably active, cheap, non-toxic and chemically stable over a wide pH range. In general, the goal of the application of photocatalysis in water treatment is the transformation, deactivation and finally mineralization of environmentally persistent compounds or xenobiotics. A large number of studies have been dedicated in our laboratory to develop transparent TiO_2-anatase films [26]. They were efficiently used to initiate the photocatalytic degradation of various organic compounds, such as pesticides and also their metabolites [27–29].

The results of our previous study [30] revealed that statin might be persistant under environmental conditions, partially being degraded in aqueous solutions, and partially converted to various, more stable transformation products. Thus, we decided to broaden our research to the field of photocatalysis of a group of possible pollutants–statins. The aim of this work was to study the photocatalytic degradation of simvastatin, lovastatin and pravastatin cholesterol-lowering statin drugs, irradiated in the presence of TiO_2 catalyst, in order to get a better insight into the mechanism of their degradation. Mass spectrometry and toxicity assessment (bioluminescence inhibition of *Vibrio fischeri* bacteria) were devoted to elucidation of the photodegradated products and to the evaluation of their toxicity activity.

2. Experimental

2.1. Materials

The statin drugs used for our research were at least 99.9% pure, so no further purification was required. Simvastatin and lovastatin (as lactones) and pravastatin (as sodium salt of active hydroxy acid form) (Fig. 1) were kindly provided by one pharmaceutical company. Hydrochloric acid, sodium chloride; sodium hydroxide and glacial acetic acid were purchased from Carlo Erba Reagents (Rodano, IT), acetonitrile (highest grade available) from Sigma–Aldrich Company Ltd (Gillingham, GB). They were used without further purification. Double de-ionized water was prepared through the Milli_Q Plus Ultra-Pure water system (Millipore, Billerica, USA) and its purity was controlled by its resistivity.

For the preparation of sol–gel derived TiO_2 films tetraethoxysilane (Acros Organics, Geel, Belgium), ethanol (Riedel-de Haen, Hanover, Germany), and concentrated (65%) nitric acid (Acros Organics, Geel, Belgium) and for TiO_2 sol: titanium (IV) isopopoxide (Acros Organics, Geel, Belgium), ethyl acetoacetate (Riedel-de Haen, Hanover, Germany), 2-methoxyethanol (Fluka, Buchs, Switzerland), ND The Triblock Copolymer Pluronic F-127 Sigma–Aldrich Company Ltd (Gillingham, GB) were used.

Transparent TiO_2-anatase films deposited on both sides of SiO_2-precoated soda lime glass slides (175 mm × 12.5 mm × 2 mm) were produced by sol–gel processing route, as described elsewhere [26]. The photocatalytic cell consisted of a DURAN glass tube (240 mm, inner diameter 40 mm), which was closed on the lower side with a glass frit and the valve for purging with oxygen. The effective volume of the glass tube was 250 mL. The spinning basket was made entirely of Teflon and fitted into the photocatalytic cell. Six glass slides with immobilized catalyst were fastened around the axis by the help of two holders. The glass slides and the axis were not joined together. There was a gap of 1.5 mm in between to enable homogenous mixing of the solution in all segments of the cell. The spinning basket with immobilized TiO_2 placed in the glass tube could freely rotate around its axis. A detailed description of the photoreactor and the cell (both developed in our laboratory) is given elsewhere [27]. The photocatalytic activity of the prepared TiO_2 films was evaluated in a tailor-made chamber photoreactor using 3 low-pressure mercury fluorescent lamps as a UVA radiation source (CLEO 20 W, 438 mm × 26 mm, Phillips; broad maximum at 365 nm). The photocatalytic cell was put in the centre, between the lamps. The motor on the top of the reactor rotates the spinning basket with the variable speeds (0–300 rpm).

2.2. Instruments and methods

The degradation of the statins and the formation of by-products were followed by HPLC system. It consisted of a HP 1090 series chromatograph, coupled with DAD detector (Agilent Technologies, Santa Clara, USA). Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm) was provided by Agilent Technologies (Santa Clara, USA). Detection was performed at 238 nm. The eluents consisted of acetonitrile (Eluent A) and grade water (pH 4.0 acidified with glacial acetic acid) (Eluent B) with flow rate 1 mL min^{-1}. The injection volume was 50 μL. Simvastatin and lovastatin were separated at the isocratic conditions as follows: 70% A, 30% B and pravastatin at 30% A, 70% B. Working solutions were prepared daily, in order to avoid hydrolysis, by diluting the stock solutions with double-deionised water.

LC–MS studies were carried out with a Waters Alliance 2695 (Milford, USA) high performance liquid chromatography system coupled to a Quattro LC triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a pneumatically assisted electrospray ionisation source (ESI) in positive ion mode and a Waters photodiode array detector. Each single experiment permitted the simultaneous recording of both UV chromatogram at a preselected wavelength and an ESI-MS full scan. The capillary voltage was set to 3.0 kV, while the sampling cone voltage was equal to 35.0 V. Source and desolvation temperature were set to 120.0 °C and 300.0 °C respectively. MS data were acquired over an m/z range 50–800 at collision energy of 10.0 eV, by MassLynx NT 3.5 Waters system. Mobile phase consisted of acetonitrile (mobile phase A) and water, acidified with acetic acid to pH 4.0 (mobile phase B) with gradient programme as follows: 0 min 5% A; 15 min 95% A; 25 min 95% A, 35 min 5% A. The flow rate was equal to 0.2 mL min^{-1} and the injection volume was equal to 30 μL.

LUMIStox system, produced by Hach-Lange (Dusseldorf, Germany) was applied for the toxicity experiments. Total organic

carbon (TOC) measurements were performed on Multi N/C 3100 Analytic Jena AG (Jena, Germany).

Transient absorption experiments in the 20 ns to 400 μs time scale were carried out on a nanosecond laser flash photolysis spectrometer from Applied Photophysics (LKS.60). Excitation (λ = 355 nm) was from the third harmonic of a Quanta Ray GCR 130-01 Nd:YAG laser (pulse width ≈5 ns), and was used in a right-angle geometry with respect to the monitoring light beam.

2.3. Photocatalytic experiments

Aqueous solutions of simvastatin, lovastatin and pravastatin at the concentration of 10 mg L^{-1} were irradiated in the presence of six glass slides with immobilized sol–gel derived TiO_2. As already described in our previous study [30] statins are susceptible to the hydrolysis under diverse pH values. Our experiment involved irradiation of lactone forms (pH 7) and open hydroxy forms (pH 9) in order to investigate whether there is any difference in their photostability. Pravastatin compound was found only as hydroxy acid regardless of pH. Besides, it has been observed that lactone forms of statin (simvastatin and lovastatin), while purged with oxygen/air/nitrogen are susceptible to evaporation. Simple experiment confirmed our hypothesis. While purging the water sample (lactone form of statin) in the plastic tube, its vapours were collected into another tube filled with 1 mL of de-ionised water and analysed on HPLC-DAD instrument. Statin compound was detected in the second tube, while in the first one its concentration decreased significantly (more than 40% after 20 min). In order to confirm that only lactone form evaporates so easily, it was converted to hydroxy acid form (by the increase of pH to the value of 9) and the experiment was repeated. No evaporation was observed for any of the investigated statins, and the compound (as hydroxy acid) could not be detected. No decrease of pravastatin concentration, as hydroxy acid, was observed.

The irradiation experiments were performed at two, diverse values of pH 7 and 9, in order to investigate if the decrease of statin concentration is attributed to its photodegradation or evaporation. Besides, for both mentioned above pH values the influence of oxygen was investigated by bubbling the sample with oxygen during the whole experiment. Since for lactones (pH 7, purged constantly with oxygen) we observed too fast disappearance (attributed to its evaporation) no rate constant could be determined. For this reason we decided to purge the sample with oxygen only for 15 min before the irradiation test took place, in order to supply sufficient amount of oxygen. For basic media (simvastatin and lovastatin as hydroxy acid) the same procedure was followed (purging and non-purging with oxygen). Pravastatin sample has been provided with oxygen, during the whole experiment, regardless of pH. Blank experiments were performed in the same manner, by irradiation compounds of interest without TiO_2 glass plates (with and without the presence of additional oxygen).

2.4. Laser flash photolysis experiments

For the laser flash photolysis experiments a 3 cm^3 volume of solution was used in a quartz cuvette, and was stirred after each flash irradiation. Individual cuvette samples were used for a maximum of 3 consecutive experiments. The transient absorbance at preselected wavelength were monitored by a detection system consisting of a pulsed xenon lamp (150 W), monochromator, and a 1P28 photomultiplier. A spectrometer control unit was used for synchronizing the pulsed light source and programmable shutters with the laser output. This also housed the high-voltage power supply for the photomultiplier. The signal from the photomultiplier was digitized by a programmable digital oscilloscope (HP54522A).

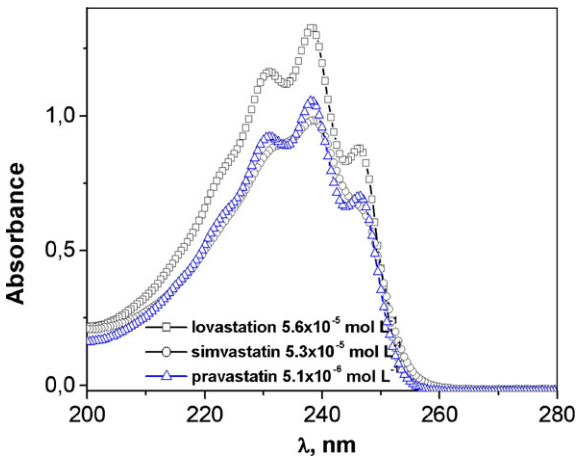


Fig. 2. Absorption spectra of lovastatin, simvastatin, and pravastatin in aqueous solution, pH 5.5.

A 32 bits RISC-processor kinetic spectrometer workstation was used to analyse the digitized signal.

2.5. Toxicity experiments

In this study the toxicity of aqueous samples for each standard statin compound and irradiated solutions (at the initial concentration of 10 mg L^{-1}; pH 7) was determined after 30 min of bacteria exposure. The toxicity categories based on the EC_{50} values, "*very toxic to aquatic organisms*" ($EC_{50} \leq 1$ mg L^{-1}), "*toxic*" (EC_{50} in the range of 1–10 mg L^{-1}), and "*harmful*" (EC_{50} in the range of 10–100 mg L^{-1}), which are established in legislation (Directive 93/67/EEC), were applied in this research to classify the target compounds [36]. Analysed samples were treated with appropriate amount of NaCl (2 g/100 mL solution) and the pH of each sample was adjusted to the value of 7 ± 0.2. Each solution was exposed to luminescent bacteria for 30 min at 15 ± 0.2 °C using a temperature-controlled block. The preserved luminescent bacteria were reactivated before the test was started. Their viability was indicated by their natural luminescence. *Vibrio fischeri* was added to each sample in two parallels and luminescence was measured for each cuvette immediately and after 30 min of incubation. Whole process was done by stable temperature 15 ± 0.2 °C and in accurate time intervals which was both provided under machine signalization.

Described above method was implemented for the toxicity evaluation of standards and degradation products (formed during photocatalysis experiment; pH 7). The luminescence inhibition for standards and irradiated samples were compared in order to investigate whether the irradiation products retain the activity of the parent molecule to elicit a toxicological effect on non-target organisms in aqueous systems.

3. Results and discussion

3.1. Kinetics

The UV absorption spectra of the studied statins in aqueous solution are given in the Fig. 2. The three of them exhibit a band, with a vibrational structure within the wavelength range 220–260 nm. The molar absorption coefficient at the maximum absorption was evaluated to 23,680 mol^{-1} L cm^{-1} and 18,490 mol^{-1} L cm^{-1} for lovastatin and simvastatin respectively and was roughly one order of magnitude higher for pravastatin (205,880 mol^{-1} L cm^{-1}). Such absorptions correspond more likely to the π–π* transition owing to the presence of the conjugated double bands. When compared

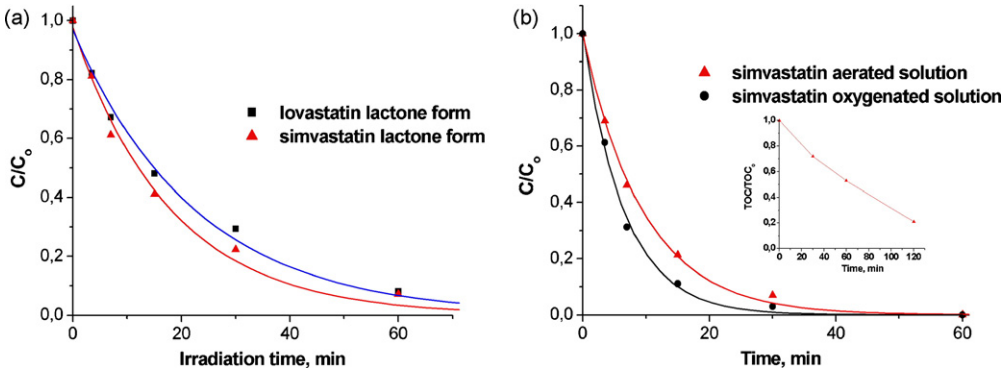


Fig. 3. Degradation curve of statin compounds irradiated in the presence of TiO_2 catalyst under diverse experimental conditions. (a) Simvastatin and lovastatin in their lactone forms under aerated conditions. (b) Effect of oxygen on simvastatin disappearance.

to simvastatin and lovastatin, the relatively high absorption coefficient in the case pravastatin is probably due to the vicinity of the OH group in molecular structure. No effect of pH within the range 2–11 was observed on the absorption spectra of the three compounds showing that both lactone and open forms present similar spectroscopic features. It has to be pointed out that the absorption spectrum does not show any overlap with the solar emission ($\lambda > 300$ nm) leading to the conclusion that the potential way for the removal of these statins from aqueous solutions must involve an Advanced Oxidation Process (AOP's).

The catalytic degradation of the statins was studied in the presence Titanium dioxide films and under light illumination at 365 nm. Under our experimental conditions, the direct photolysis of statins was not involved and the adsorption on TiO_2 films was clearly negligible. As shown in the Fig. 3a and b, under our experimental conditions, the degradation of the simvastatin and lovastatin in their lactone forms was very efficient.

The degradation curves were nicely fitted using apparent first order kinetics. The observed rate constants were roughly similar for both compounds (Table 1). The kinetics of degradation was studied as a function of various experimental conditions, such as pH and the amount of dissolved oxygen. The former parameter permitted to presence of either open hydroxy or closed lactone forms of statins (except for provastatin which is only present in hydroxy form) and the latter parameter is essential for the photocatalytic process to occur. Its presence in the medium permits the scavenging of the electron leading to the formation of superoxide anion ($O_2^{\bullet -}$) and an efficient formation of hydroxyl radical through the oxidation of water molecules by the hole h^+ [27–29]. The generated superoxide anion may also act as a source of hydroxyl radical on TiO_2 surfaces. Depending on the experimental conditions, the total disappearance of the statins, followed by HPLC measurements, was reached after about 60 min irradiation time. Table 1 gathers all the values of the rate constants for various experimental conditions (pH and oxygen concentration).

For simvastatin and lovastatin bubbled with oxygen for 15 min before the experiment, and for pravastatin purged during the whole experiment, the observed rate constants were evaluated to: $0.052 \pm 0.008\ min^{-1}$; $0.046 \pm 0.006\ min^{-1}$ and $0.107 \pm 0.006\ min^{-1}$ respectively. The total organic carbon measurements for simvastatin (Fig. 3) showed that an efficient mineralization was obtained (79% TOC conversion in 120 min). Such mineralization clearly indicates that statins as well as the generated products are efficiently degraded under our experimental conditions. When present in the hydroxy acid forms, a faster degradation was obtained as shown in Table 1. At the same time we noticed that for hydroxy acids (pH 9) bubbled with oxygen during the whole experiment higher degradation rates are observed than for non-purged samples. This clearly demonstrates the main role of oxygen in the irradiation process. Open hydroxy acids (simvastatin and lovastatin) appear to be more susceptible to the degradation than their corresponding lactones.

3.2. Product analysis

The LC–MS analysis of all three compounds showed the formation of several irradiation products. Unfortunately, the MS data did not provide more detailed structural information to determine the position of the added hydroxyl group on the statin ring, so that several isomers can be hypothesized for this compound. Often, single MS turns out not to be a sufficient measurement to conclude with absolute certainty identity of formed transformation products. Additional analysis would be required (LC-nuclear magnetic resonance, chemical derivatisation, and hydrogen/deuterium-exchange (H/D-exchange) combined with MS), to define exact structure of proposed isomers.

For the presentation of photogenerated products, we have selected one representative of statin drugs – simvastatin since similar behaviour of all investigated statins was observed and by-products are formed following identical degradation pathway. LC–MS analysis confirms that formation of irradiation products of

Table 1
Observed rate constants for the photocatalytic degradation of statins under various experimental conditions.

	k_{obs} (min^{-1})			
	Closed lactone form (pH 7)		Open hydroxy acid form (pH 9)	
	[a]	[b]	[a]	[b]
Simvastatin	n.d.	0.052 ± 0.008	0.113 ± 0.007	0.095 ± 0.006
Lovastatin	n.d.	0.046 ± 0.006	0.105 ± 0.004	0.089 ± 0.003
Pravastatin	–	–	0.107 ± 0.006	0.083 ± 0.004

n.d., not detected. The determination of rate constant was not possible due to the fast evaporation of lactone forms.
[a] Sample bubbled with oxygen for the whole duration of the irradiation.
[b] Sample bubbled with oxygen only for 15 min before the irradiation.

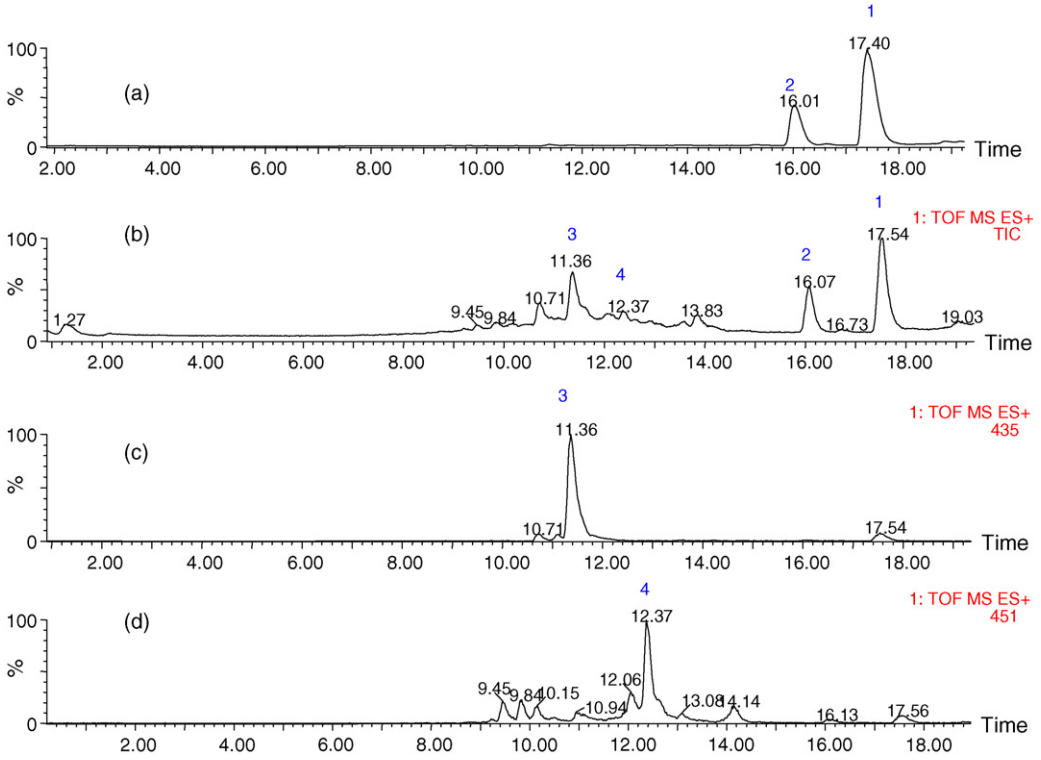


Fig. 4. LC–MS total ion chromatogram of standard simvastatin sample (a); 15 min irradiated sample in the presence of TiO_2 catalyst (b); extracted mass chromatogram of irradiated sample at *m*/*z* 435 (c) and at *m*/*z* 451 (d).

all the investigated statins appear through the hydroxyl radical attack on the double bond of the ring. Total ion chromatogram of simvastatin sample (irradiated for 15 min) is presented on the Fig. 4b. The irradiation time of 15 min corresponds to the time, where the concentration of the generated products is the highest. Prolonged irradiations lead to their disappearance. As can be seen in Fig. 4b, the initial compound is still present in two forms: lactone (ret time 17.54 min) and hydroxy acid (ret time 16.07 min). At least two hydroxyl isomers have been confirmed for simvastatin by the extracted chromatogram at *m*/*z* 435 – peaks with retention time 10.71 min; 11.36 min (Fig. 4c). Subsequently the molecule is oxidized and $HO_2^{\bullet}$ radical is eliminated, resulting in conjugated double bond reconstruction (giving the mentioned above hydroxyl isomers, as an intermediate products). Table 2 presents suggested photogenerated simvastatin photo-products and its most important fragment ions, tentatively elucidated by studying their MS spectra in positive mode. Besides, main fragment ions, detected previously for standard sample (*m*/*z* 303, 285, 267) are present in the hydroxylated molecule, with the addition of mass of 16 (giving *m*/*z* 319, 301, 283). Such findings clearly demonstrate that oxidation reaction occurred leading to the formation of M+16 products (hydroxylated derivatives). Their presence has been confirmed by the total ion chromatogram after 3.5 min of irradiation experiment and their amount increases with irradiation time. At 15 min the concentration of those M+16 products has reached maximum, and parallelly to them, dihydroxylated derviatives (M+32) were formed in the solution. Irradiation in the presence of TiO_2 catalyst results in dihydroxysimvastatin derivative product. Ions with *m*/*z* 451 (extracted from total ion chromatogram, Fig. 4d), are the confirmation of proposed structure. Other, major fragments of the irradiated product (dihydroxylated simvastatin) are *m*/*z* 433, 335, 317 and 281 (see Table 2).

Taking into account the obtained results, the proposed mechanism is suggested and explained in details in the Fig. 5.

After 120 min of catalysed irradiation (simvastatin and lovastatin purged with oxygen only before the experiment), and 60 min (pravastatin; purged during the whole irradiation) parent compounds are not observed anymore in the reactivation solution.

3.3. Laser flash photolysis studies

From the previous results, we clearly demonstrated that hydroxyl radicals, produced from titanium dioxide, are the key species in the transformation of statins. Thus we decided to undertake a nanosecond laser flash photolysis study in order to evaluate the second order rate constant of such reactivity. The production of hydroxyl radicals was obtained using the iron(III) aquacomplex, namely $[Fe(OH)(H_2O)_5]^{2+}$. The advantage of such complex is its efficient production of hydroxyl radical through a ligand to metal charge transfer (LMCT) process upon excitation with a third harmonic from a Nd:YAG laser (355 nm) [31–35].

$$[Fe(OH)(H_2O)_5]^{2+} \xrightarrow[LMCT]{h\nu} Fe^{2+} + \cdot OH + 5H_2O$$

When the mixture statin/iron(III) (1.0×10^{-4} mol L^{-1}/3.0×10^{-4} mol L^{-1}) is flash photolysed at 355 nm, no build-up of the absorbance was observed within the region 300–600 nm.

However a depletion of the absorbance is observed when the detection was performed at $\lambda < 350$ nm (Fig. 6a). This is due to the instantaneous disappearance of the iron (III) species via the LMCT process as previously stated [34]. Such observation also leads us to the conclusion that the intermediate formed by the reaction of the hydroxyl radical with the statin does not absorb within our detection wavelength range. This is in complete agreement with the chemical structure since no chromophore units are present.

In order to determine the absolute second order rate constant of the reaction between the hydroxyl radical and the statins, competition kinetics using metsulfuron methyl (MTSM)

as a competitor, were undertaken. MTSM was chosen because its reactivity toward hydroxyl radicals is well known, with a rate $k_{MTSM} = 3.5 \times 10^9$ mol L^{-1} s^{-1}. The reaction leads to an intermediate, namely •OH-MTSM adduct. Using laser flash photolysis setup, the intermediate can be easily detected under our experimental conditions ($\lambda_{max} = 350$ nm) [34]. Its growth showed a pseudo-first order kinetics (Fig. 6b). In the presence of various concentrations of statin, the formation rate appeared to increase as a consequence of competitive reactions (Fig. 6c).

When the mixture iron (III)/statin/MTSM is used the following processes occur:

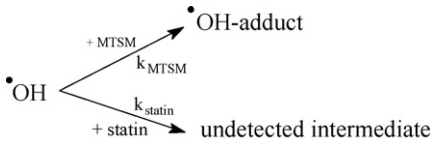
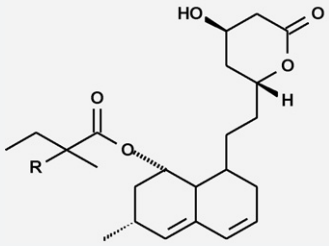
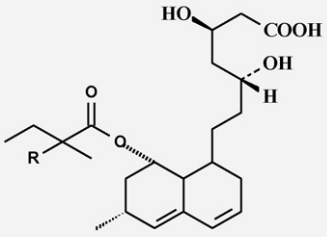
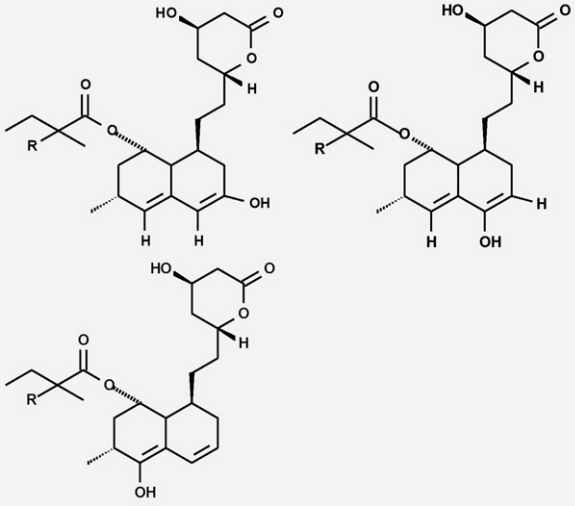
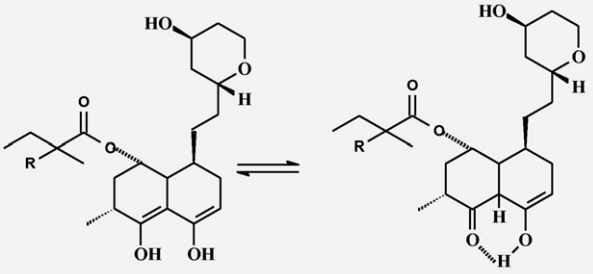


Table 2
LC–MS data in ES positive mode for the simvastatin photogenerated products (in the presence of TiO_2 catalyst), and their proposed structures.

Product[a]	t_{ret}[b]	[M+H]+; main fragment ions	Structure
1	17.5	419, 303, 285, 267, 243, 225, 199	
2	16.0	437, 303, 285, 267, 243, 225, 199	
3	10.71 and 11.36	435, 319, 301, 283, 241	
4	12.37	451, 433, 335, 317, 281	

[a] The numbers refer to the peaks in LC–MS chromatogram given in Fig. 4.
[b] Retention time.

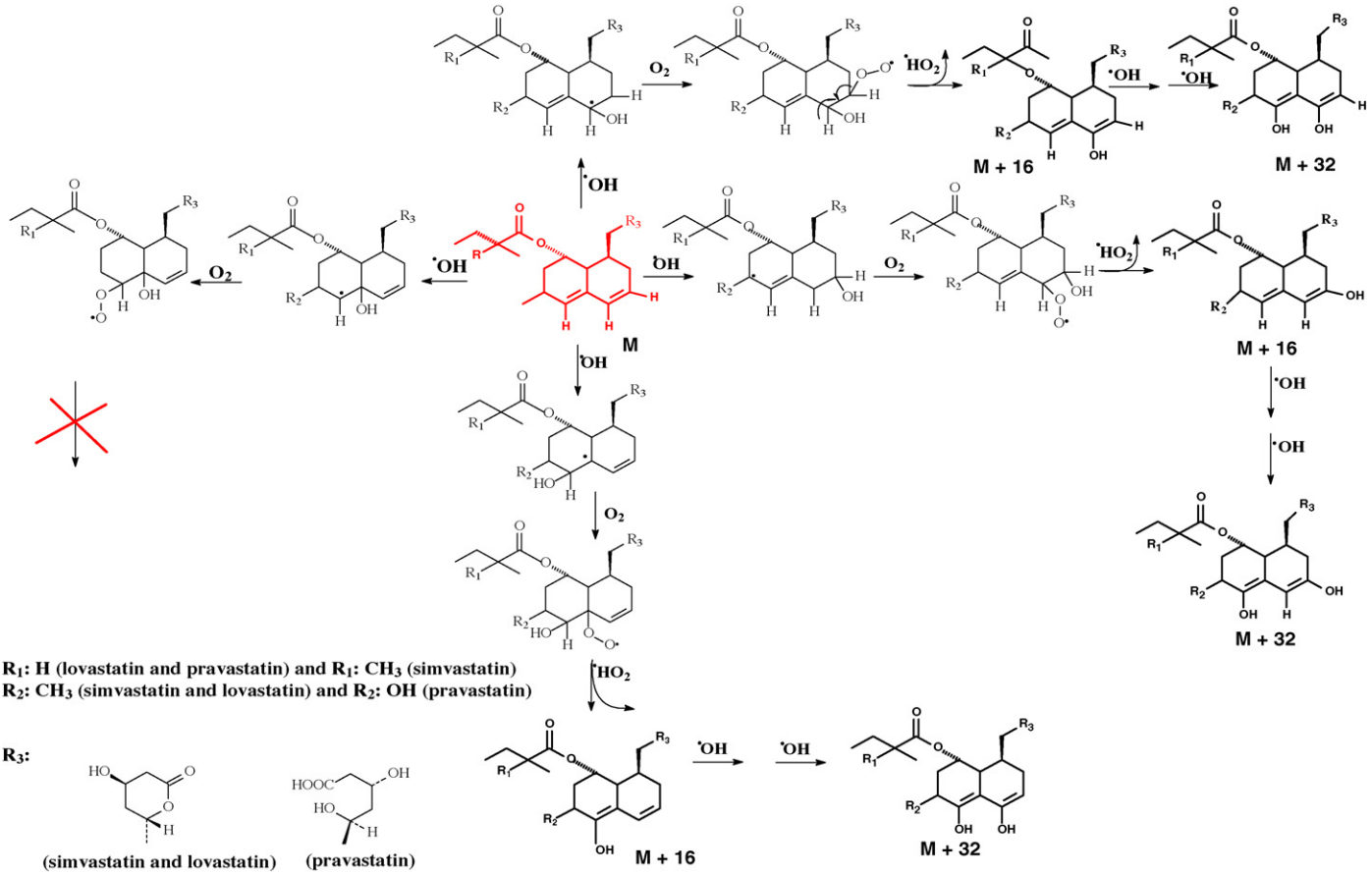


Fig. 5. Proposed photodegradation pathway of statin compounds in the presence of TiO_2 catalyst.

From the above competitive reactions one can deduce the observed rate constant (k_{obs}) for the formation of $^{\bullet}$OH-MTSM arising from MTSM as:

$$k_{obs} = k_{MTSM}[MTSM] + k_{statin}[statin]$$

The plot of k_{obs} as a function of statin concentration will lead directly to k_{statin} as the slope and $k_{statin} \times [statin]$ as the intercept. The experiments were undertaken by excitation at 355 nm using mixtures of iron(III) complex (3.0×10^{-4} mol L^{-1})/MTSM (1.0×10^{-4} mol L^{-1})/various concentrations of pravastatin (from 2.0×10^{-4} to 6.0×10^{-4} mol L^{-1}). As clearly shown in the Fig. 7, the plot k_{obs} as a function of lovastatin concentration is linear, in complete agreement with the kinetic expression. The slope $k_{pravastatin}$ was evaluated to 8.6×10^{8} mol L^{-1} s^{-1}. For the two other statins, because of the low solubility, the rate constants could not be determined with sufficient precision. Using a method similar to that performed with pravastatin, they were evaluated to be about 4×10^{8} for both, simvastatin and lovastatin.

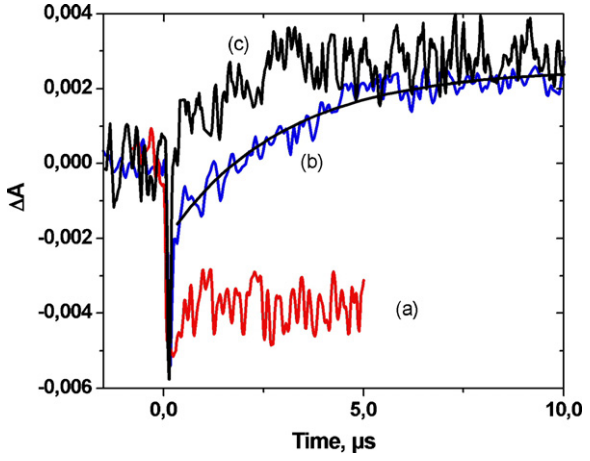


Fig. 6. The evolution of the absorbance at 350 nm as obtained by laser flash photolysis ($\lambda_{excitation}$ = 355 nm). (a) Mixture pravastatin/iron(III) (1.0×10^{-4} mol L^{-1}/3.0×10^{-4} mol L^{-1});(b) mixture MTSM/iron(III) (1.0×10^{-4} mol L^{-1}/3.0×10^{-4} mol L^{-1}); (c) mixture MTSM/iron(III)/pravastatin (1.0×10^{-4} mol L^{-1}/3.0×10^{-4} mol L^{-1}/3.0×10^{-4} mol L^{-1}).

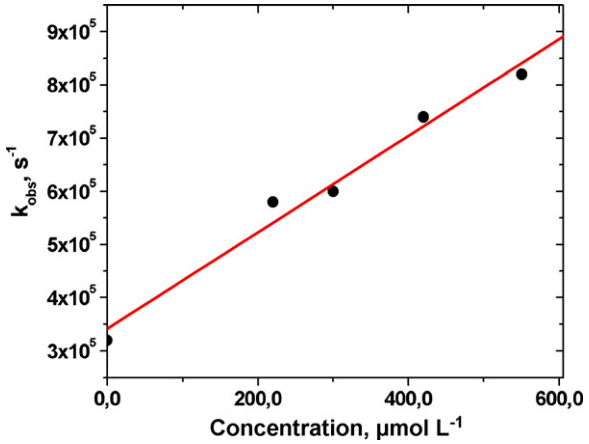


Fig. 7. The evolution of k_{obs} as a function of pravastatin concentration.

Table 3
Experimental data of luminescence inhibition of statin standard solutions as well as irradiated samples.

	Luminescence inhibition of *Vibrio fischeri* (%)	
	Standard sample solution before irradiation	Sample after irradiation
Simvastatin	9.8 ± 1.4	13.5 ± 2.9
Lovastatin	10.4 ± 2.1	9.2 ± 2.7
Pravastatin	8.2 ± 2.3	9.3 ± 2.2

3.4. Toxicity of irradiated samples

Comparison of bioluminescence inhibition for standards and photogenerated products (the highest concentration of statin derivatives during sample irradiation: 15 min irradiation of simvastatin and lovastatin; 7.5 min for pravastatin; in all cases pH 7) reveals that transformation products do not possess higher toxicity than initial compounds (Table 3). Toxicity of photogenerated products has shown (13.5 ± 2.9)% inhibition for simvastatin, (9.2 ± 2.7)% for lovastatin and (9.3 ± 2.2)% for pravastatin, which does not indicate harmful effect towards *Vibrio fischeri*. Applied initial concentration of 10 mg L^{-1} was of the maximum water-solubility, and is far above the expected environmental occurrence (ng L^{-1}). Thus, none of these chemicals can be considered as toxic, since inhibition effects of 50% were not observed and their respective EC_{50} values would exceed the maximum concentration in which they are soluble in water.

These preliminary toxicity investigations are the evidence that during catalysed irradiation procedure non-toxic (in regard of *Vibrio fisheri* bacteria) compounds are produced, which is very important from the environmental point of view, in the case of catalysed irradiation method implementation as a tool for statin removal. Further tests should involve broader range of tested organisms.

4. Conclusions

On the basis of obtained results, one can conclude that photocatalytic degradation is a complex process, involving various intermediate products (isomers). The faster disappearance for simvastatin and lovastatin in lactone form (in comparison to open hydroxy forms) is attributed to its evaporation. When pH was converted to basic (lactones of simvastatin and lovastatin were transformed to hydroxy acids) similar degradation rate as previously observed for pravastatin was noticed. Within 120 min complete removal of simvastatin and lovastatin initial compounds was achieved, regardless of pH. Additional experiments, where oxygen was replaced with air showed that more time is required to degrade completely statin compounds, and that oxygen plays an important role in the catalysed degradation. The rate constant for the reaction of the hydroxyl radical with the statins was determined by means of laser flash photolysis experiments and by employing competitive reactions: $k_{pravastatin} = 8.6 \times 10^8$ mol L^{-1} s^{-1}.

LC–MS analysis confirms that formation of irradiation products appear through the hydroxyl radical attack on the double bond of the ring. The difference in the mass of major fragments (16) suggests the hydroxylation mechanism and degradation through hydroxyl radicals attack (presence of the additional OH group). M+32 is attributed to two, additional OH groups in the statin molecule (difference of 32 in mass of initial compound), giving dihydroxylated derivatives. Unfortunately, the MS data did not provide more detailed structural information to determine the position of the added hydroxyl group on the statin ring; so that several isomers can be proposed for these compounds (vicinity of conjugated double bonds is favourable). Hence, all the possible isomers were taken into account.

Statin degradation is a challenging task, pointing out catalysed irradiation as a one of the methods of statin removal, in the case of their presence in the sewage treatment plants. It is of great importance that toxicity test proved non-toxicity of formed products, where *Vibrio fischeri* bacteria were used as preliminary tested organisms. Thus, we strongly believe that possible methods for statin degradation should be further investigated.

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