

Photodegradation of Atorvastatin under Light Conditions Relevant to Natural Waters and Photoproducts Toxicity Assessment

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Abstract

Atorvastatin, widely prescribed hypolipidemic drug, undergoes rapid, probably self-sensitised, degradation (less than 20% left after 25-minute irradiation) if irradiated by wavelengths 300 - 350 nm in aqueous solution. When ferric ions are added to the reaction mixture, the degradation follows first order kinetics with a rate constant of 0.130 min^{-1} . Photochemical degradation may thus represent a significant way of environmental transformation of this pharmaceutical. Toxicity testing of atorvastatin and atorvastatin photoproducts performed on the water plant *Lemna minor* revealed that atorvastatin itself exhibited no observable toxic effect measured as leaf area growth inhibition, while the photoproducts showed a significant toxicity to the plant, which shows the extreme importance of investigating not only toxicity of drugs themselves on aquatic organisms but also effects of their transformation products.

Keywords

Atorvastatin, Photodegradation, Atorvastatin and Photoproducts Toxicity, *Lemna minor*

1. Introduction

Pharmaceuticals for human use have recently received much attention as emerging organic micropollutants in the aquatic environment [1] [2] [3] [4] [5]. These compounds enter the aquatic environment via waste-water treatment plants as a result of the incomplete removal of these compounds from hospital and manufacturing plant effluents and domestic wastewater.

The effort of developers and manufacturers is focused on the stability of

pharmaceuticals to prolong their shelf-life. Thus these compounds are in most cases resistant to hydrolysis and biodegradation, and a photoinitiated degradation represents possible transformation pathways in the aquatic environment. Photoinitiated reactions in sunlit surface waters can proceed as 1) direct photodegradation (with compounds absorbing high-energy solar radiation reaching the Earth's surface), 2) indirect reactions mediated by photochemically formed intermediates such as hydroxyl and other radicals, singlet oxygen, aquated electrons [1] [6] [7] or 3) photocatalysed reactions in which the active forms of catalysts are formed photochemically [2].

Atorvastatin is a member of the group known as statins, antilipidemic drugs that lower the level of cholesterol in the blood by reducing cholesterol production by the liver [8]. Statins belong to the most widely prescribed drugs in developed countries and have been detected in natural waters worldwide in concentrations ranging from 10 ng/l to 1500 ng/l [9] [10] [11] [12]. Their photochemical degradation has been studied by several scientific groups. Lam & Mabury [1] Lam *et al.* [13] and Wang *et al.* [14] studied the influence of dissolved organic matter and some inorganic ions on atorvastatin photochemical degradation, while Razavi *et al.* [7] and Wang *et al.* [15] investigated the effect of reactive oxygen species on the photodegradation of atorvastatin.

The presence of statins in natural surface waters provoked studies on their possible toxicity to water organisms such as fish [16] [17], mussels [18], or crustaceans [19].

The objective of this study was firstly to investigate the photocatalytic role of ferric ions in the photoinitiated transformation of atorvastatin and secondly to test the toxicity of atorvastatin itself and its photoproducts on the water plant *Lemna minor*. The motivation for the study was the fact that toxicity of drugs repeatedly detected in the aquatic environment is investigated but there is an absolute lack of information about toxic effects of compounds formed during the transformation of the drugs in the aquatic environment.

2. Materials and Methods

Atorvastatin (more than 99.9%) was purchased from Merck (Germany), FeCl₃ and HCl (both p.a.) from Lachema (Czech Rep.). Milli-Q® water was used for the dissolution of chemicals.

Irradiation: Samples containing 3 ml of the atorvastatin solution in 1-cm glass cuvettes with PTFE lids were irradiated in a Rayonet reactor with RPR 3000 Å lamps emitting light at a wavelength range of 254 - 350 nm, light below 300 nm was filtered out by optical glass to imitate the short-wavelength solar radiation that reaches the Earth's surface. Experimental apparatus used for irradiation is illustrated in **Figure 1**. Radiant flux was measured using a Lutron UV A light metre, the total power of all of the electromagnetic radiation within the wavelength range 320 - 390 nm (the range detected by the Lutron metre) emitted per unit time was calculated for the irradiated area, the value being 4.5 W.



Figure 1. Irradiation of samples in Rayonet reactor with RPR 3000 Å lamp. The cuvettes with irradiated samples were placed on an aluminium stand at a height in the middle of the lamps length. Behind the lamps, there is a lining of burnished aluminium serving as a reflecting mirror.

Analyses of samples: The extent of atorvastatin photodegradation was determined by HPLC (ThermoScientific Dionex Ultimate systém 3000 (USA), column Phenomenex Kinetex® 5 mm EVO C18, 30 × 2.1 mm, mobile phase water with 0.001% HCOOH and acetonitrile 0.6:0.4 (v/v) with PDA 3000RS spectrophotometric and FLD 3000RS fluorescence detectors.

The extent of ferric ions reduction during irradiation was determined spectrophotometrically using 1, 10-phenantroline as a complexing agent for ferrous ions.

A *Lemna* sp. growth inhibition test was performed according to the OECD Guideline [20] using the vascular plant *Lemna minor* which was obtained from a natural water reservoir in Netolice in the southern part of Czech Rep. (GPS coordinates 49°02'53"N; 14°12'30"E). Prior to testing, the plants were cultivated in Swedish Standard *Lemna* sp. growth medium (SIS) prepared accordingly to OECD (2002) for 3 weeks. To test the effect of atorvastatin on the *Lemna* growth, a concentration series of 0, 25, 50, 100 and 200 mg/l was prepared in SIS medium. All growth inhibition tests were performed in opened glass crystallising dishes (Ø 9 cm), under natural daylight and at 23°C temperature for seven days. Colonies consisting of cca 10 fronds (leaf surface area approx. 1 cm²) were exposed to the concentration series of atorvastatin; each concentration treatment was carried out in 4 replicates. The total frond area was measured using the Easy Leaf Area software [21]. To test the effect of photoproducts, a mixture of photodegradation products (irradiated sample, irradiation time 15 minutes) was diluted to provide the final concentration of remaining atorvastatin of the same value as was its highest concentration in atorvastatin toxicity test, *i.e.* of 200 mg/l. Experimental arrangement for toxicity test is schematically shown in **Figure 2**. As illustrated in the scheme in **Figure 2**, the dishes with individual concentrations were distributed in a way to minimise systemic error based on position relative to the light source (window).

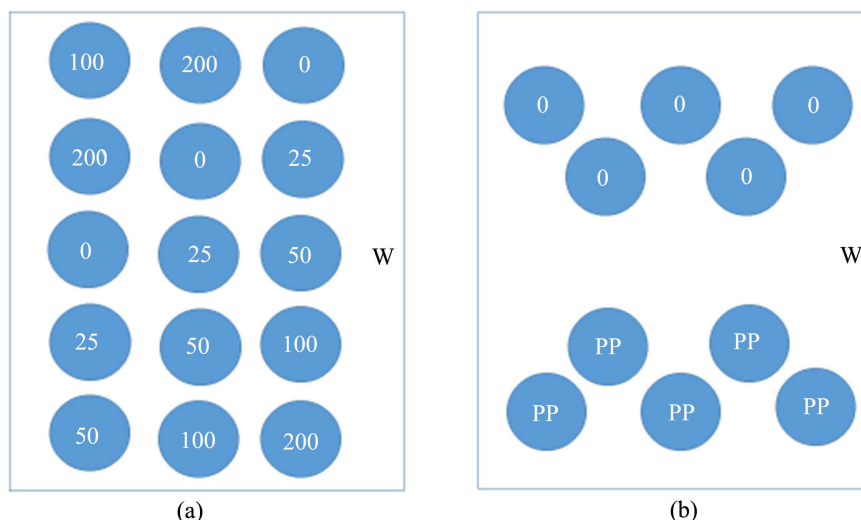


Figure 2. Experimental arrangement of toxicity test. (a) Atorvastatin toxicity test: blue circles represent individual crystallizing dishes, numbers give the concentration of atorvastatin; (b) Photoproduct test: blue circles represents individual crystallizing dishes, upper rows are control dishes (zero concentration of photoproducts), lower rows are dishes with photoproducts (PP). W shows where light source (window) was placed.

Differences between samples were evaluated using 2-way analysis of variance followed by Tukey post-hoc test; p-values less than 0.05 are considered significant.

3. Results

The absorption spectra of atorvastatin itself and of a complex of atorvastatin with ferric ions are shown in **Figure 3**. As can be seen from the absorption spectra, the atorvastatin absorption of radiation above 300 nm is very small. The addition of ferric ions results in the formation of a complex with a significantly higher absorbance in the UV region, namely with considerable light absorption in the region of wavelengths longer than 300 nm.

The photochemical degradation of atorvastatin is demonstrated in **Figure 4**. The figure illustrates that the degradation proceeds even for atorvastatin itself when no ferric ions are added, and that the degradation is rapid—more than 80% of the original amount is transformed after 25-minute irradiation. Nevertheless, the atorvastatin degradation in this case is slow in the first minutes of irradiation, the reaction rate significantly increasing after 10 minutes of irradiation. The reaction does not follow first order kinetics. With lower concentrations of added ferric ions (1.7 mg/l and 3.4 mg/l), the first slower stage of the reaction is gradually less pronounced but still noticeable. In the presence of the highest tested concentration of added ferric ions, 5 mg/l, the reaction follows first order kinetics with a rate constant of 0.130 min^{-1} . The concentration of added ferric ions did not dramatically affect the remaining amounts of atorvastatin after a 25-minute irradiation which were 13%, 9%, 7%, and 4% of the original amount for 0, 1.7, 3.4, and 5 mg/l, resp. What was remarkably influenced by the presence

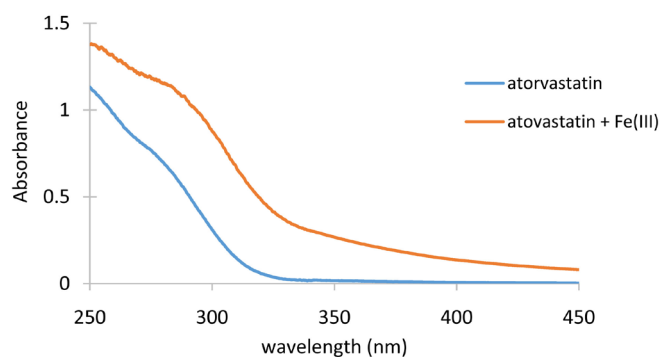


Figure 3. Absorption spectra of atorvastatin and atorvastatin with ferric ions. Concentration of atorvastatin 36 mg/l, concentration of Fe (III) 5 mg/l.

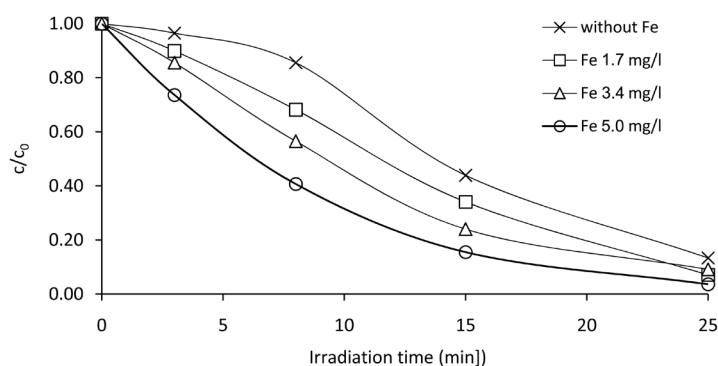


Figure 4. Dependence of atorvastatin degradation on the concentration of ferric ions added to the reaction mixture.

of ferric ions was product formation—in the presence of ferric ions significantly greater amounts of products were formed, as illustrated in **Figure 5**. **Figure 5** shows the chromatograms received with fluorescent detector where peak of atorvastatin itself (at the retention time 6.7 min.) is rather small. On the other hand, products of photochemical degradation had low UV absorption but their fluorescent signals were significant. Therefore, for evaluation of atorvastatin decay, UV detection was applied while photoproduct formation was detected using fluorescence signal. Nevertheless, as can be seen from **Figure 5**, if Fe (III) is present in the reaction mixture, even atorvastatin exhibits higher fluorescence. The two products formed in the reaction mixture where only atorvastatin was present (peaks with retention times 9.5 and 10.3) were formed as well in the presence of Fe (III), the presence of Fe (III) caused a formation of many more products.

The testing of the photochemical reduction of ferric ions in irradiated reaction mixture with 5 mg/l Fe (III) revealed that after 1 minute of irradiation, approximately 6% of added ferric ions were reduced to ferrous ones, the amount did not rise during further irradiation.

The toxicity testing of atorvastatin on *Lemna minor* was performed with the concentration of 0, 25, 50, 100, and 202 mg/l of atorvastatin. The percentage of increase in leaf area after a 7-day period incubation is illustrated in **Figure 6**.

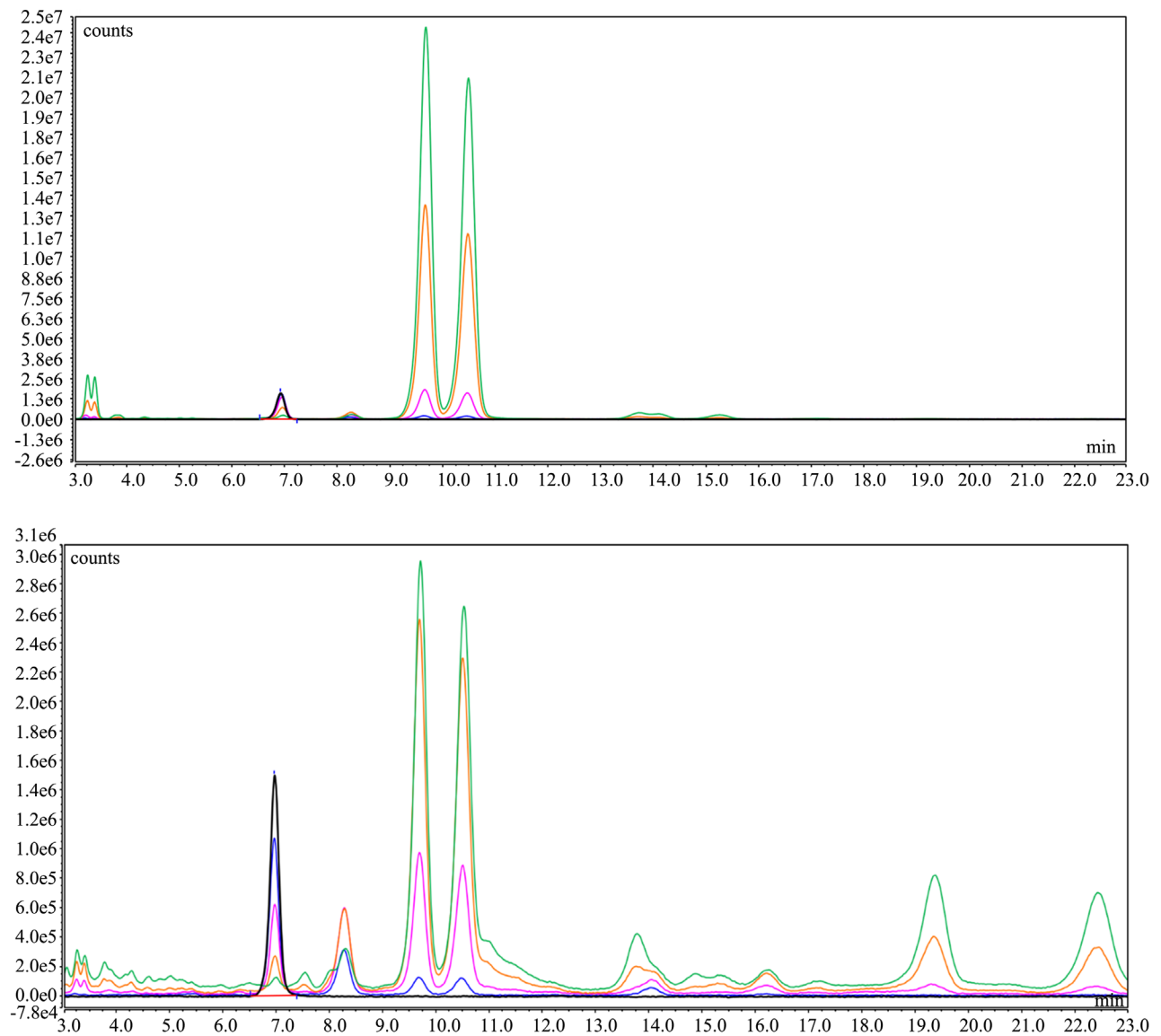


Figure 5. Comparison of the number of products formed during irradiation: upper part—atorvastatin without added Fe (III); lower part—atorvastatin + 5 mg/l Fe. Black—before irradiation; blue, pink, orange and green—irradiation of 3, 8, 15 and 25 minutes, resp.

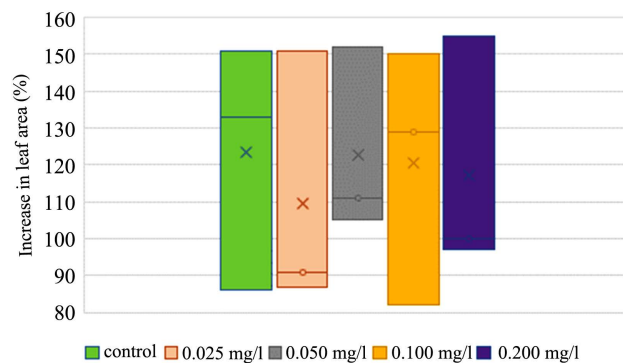


Figure 6. *Lemna minor* growth inhibition test—exposure to atorvastatin in concentrations of 0, 25, 50, 100 and 200 mg/l.

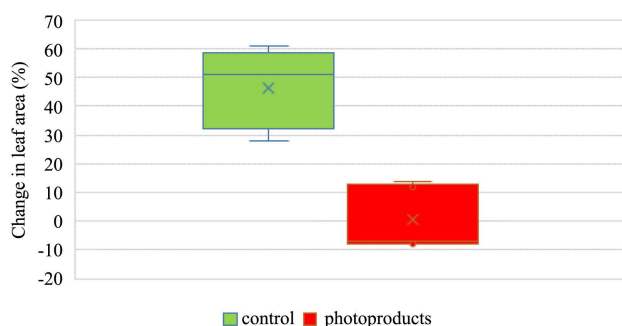


Figure 7. *Lemna minor* growth inhibition test—exposure to photoproducts of atorvastatin; concentration of remaining atorvastatin in the reaction mixture 200 mg/l.

Though the increase in leaf areas varies within individual tested concentration, the graphical representation shows no noticeable effect on *Lemna* sp. growth.

The toxicity testing of the mixture of products formed during the photochemical degradation of atorvastatin is depicted in **Figure 7**. As can be seen from the figure, there is a significant difference between the control set and the set exposed to photoproduct mixture. In the series of five control tests, the increase in leaf area varied from 28% to 61%; in the series of photoproduct tests, a small increase in two cases and even a decrease in leaf area in three cases were observed. The received value of p-parameter for the statistical comparison of the two sets of data (control vs. photoproducts) was 0.005.

4. Discussion

The degradation of atorvastatin itself in this study did not proceed as first order kinetics reaction as can be seen in **Figure 4**, though Wang *et al.* [14] reported first order kinetics with a rate constant of 0.0369 hour^{-1} for atorvastatin photochemical degradation in phosphate buffer. In our study, the reaction was slow in the initial stages of irradiation and its reaction rate increased gradually with increasing irradiation time. A similar observation was made with the photochemical degradation of another drug, verapamil, which is a calcium channel blocker [2]. A slow reaction at the beginning of the irradiation period is not surprising because of the low absorption of atorvastatin of the incident radiation. Since the reaction was performed in Milli-Q® water, catalysis by trace amounts of metal ions is not very likely. We therefore hypothesise that in the initial phase of photodegradation a product with photosensitising properties is formed, and that after an accumulation of the product, the reaction rate gradually increases due to a process of self-sensitisation [22]. Wang *et al.* [14] also suggested a possible self-sensitisation in connection with products of the photodegradation of atorvastatin with regard to the excited triplet state of atorvastatin may contribute to singlet oxygen production from the oxygen dissolved in the solution.

The relatively fast direct photochemical degradation of atorvastatin itself observed in this study (**Figure 4**) justifies an assumption that photochemical processes may significantly contribute to the transformation of the drug in surface waters exposed to solar radiation.

Addition of metal, Fe (III), ions to the reaction mixture resulted in increase in initial reaction rate as illustrated in **Figure 4**. Wang *et al.* [14] also studied the effects of various additives, among them ferric ions. In their experiments, the addition of ferric ions corresponding to the lowest concentration used in our study led to an almost eightfold increase in reaction rate, the rate constant was obtained from the first-order kinetics model. In our study, the addition of ferric ions shortened the initial “lag” phase, but first-order kinetics was a suitable model only for the highest iron concentration used in the study. The observed value of 0.130 min^{-1} differs in order of magnitude from the values reported by Wang *et al.* [14]. This variance might be caused by the different wavelength range and intensity of the used radiation since Wang *et al.* [14] gave only the intensity at one wavelength, namely 365 nm.

The presence of metal ions noticeably influenced the formation of products. In the reaction mixture of the atorvastatin alone, only two main products were created as demonstrated in **Figure 5**. In the reaction mixtures with added ferric ions, a number of other products appeared, all of them had longer retention times than atorvastatin, which means they are less polar than atorvastatin itself. Products formed in the study exhibit a significant fluorescence but they could not be detected through measuring a UV signal.

The influence of Fe (III) on photodegradation rate of atorvastatin is particularly important from environmental point of view since ferric ions are present in most surface water bodies, often abundantly, in mg/l [23]. Thus the photodegradation may provide a wide range of photoproducts as observed in this study, with consequences to toxicity (**Figure 7**).

As given in results, the testing of the photochemical reduction of ferric ions in irradiated reaction mixture with 5 mg/l of Fe (III) revealed that a very short (1 minute) period of irradiation is enough for reduction of nonnegligible amount of ferric ions (6%). The longer irradiation did not affect the amount of reduced form which means that this is the steady state concentration resulting from two processes going against each other—photochemical reduction of metal oxidised form and its reoxidation by molecular oxygen present in solution. Comparison with values reached in the photodegradation of hydrocortisone, b-estradiol, parabens [2], and verapamil [24] where the values of the ferrous ions reached 60% - 70% of the added amount led us to conclude that the photoinitiated degradation of atorvastatin did not proceed via a photocatalytic mechanism in which the reduced metal species is the active catalytic form [25]; the more likely cause of the increase in reaction rates in the initial phases of photodegradation was the direct photodegradation due to enhanced light absorption by the atorvastatin-Fe complex.

The toxicity of atorvastatin on *Lemna minor* was not proven even for concentrations as high as 200 mg/l as demonstrated in **Figure 6**, though Brain *et al.* [26] observed a toxic effect on *Lemna sp.*, namely *Lemna gibba* and determined the EC_{10} value to be 26 mg/l. On the other hand, the toxicity of atorvastatin photoproducts was conclusively demonstrated (**Figure 7**)—the received p-parameter

value of 0.005 strongly suggests that the null hypothesis on the non-toxic effect of photoproducts when compared to sets of control data is incorrect. The present study did not focus on atorvastatin products characterisation and identification and thus it cannot be concluded whether only one of the products was responsible for the toxic effect or whether it was a cumulative effect of a number of substances created by the phototransformation of atorvastatin. It would definitely be worth further investigation. It is worth mentioning that there are numerous data about toxicity of drugs on various aquatic organisms but there is a lack of knowledge of the photodegradation processes that may often (with recalcitrant molecules to which drugs belong) represent the main path of their environmental transformation. Moreover, the products of photochemical degradation may possess even more toxic capacity to aquatic organisms than the original drug themselves.

5. Conclusions

Despite the weak absorption of radiation of wavelengths longer than 300 nm, atorvastatin underwent significant degradation even without catalysing or sensitising additives. The course of degradation with a “lag” phase at the beginning of the reaction and increase in rate in its latter stages is supposed to arise from a sensitising agent formation, *i.e.* via self-sensitisation process. The finding supports a conclusion that atorvastatin may undergo direct phototransformation in the aquatic environment.

The addition of ferric ions changed the courses of photodegradation curves shortening the initial “lag” phase. With a concentration of ferric ions of 5 mg/l, the reaction followed first-order kinetics with the rate constant of 0.130 min⁻¹. The effect of ferric ions seems to be partially connected with the enhanced absorption of radiation in the range of 300 - 350 nm, partially other mechanisms may participate. Increase in atorvastatin photodegradation rate together with formation of a high number of additional product when compared to the reaction mixture without added Fe (III) leads to an assumption that the Fe-assisted photochemical degradation may be prevalent in atorvastatin environmental transformation. Reaction mixture of atorvastatin photoproducts formed in the presence of Fe (III) was conclusively shown to have notable toxic effect on the aquatic plant, *Lemna minor*. The products of atorvastatin photochemical transformation may have similar effects on other aquatic organisms as well and therefore be of high environmental significance.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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