Aqueous photocatalytic oxidation of prednisolone

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Abstract: The research into the aqueous photocatalytic oxidation of the anti-inflammatory drug prednisolone was undertaken with P25 titanium dioxide (Evonik) and visible light-sensitive sol-gel synthesized titania-based photocatalysts containing carbon, sulphur, and iron. Possible prednisolone photocatalytic oxidation reaction pathways were proposed based on a number of oxidation by-products determined in the present study. The prednisolone adsorption properties, effects of initial prednisolone concentration, pH, usual wastewater matrix admixtures, like carbamide and sucrose, were studied. The nontoxicity of doped catalysts towards Tetrahymena thermophila, a ciliate protozoa present in the activated sludge, indicated their lower oxidative ability compared to P25, but also implied their potential application in pre-treatment of toxic hazardous materials under VIS or solar radiation before the biological degradation stage.

Keywords: Photocatalysis • Corticosteroid • Doped titania • Oxidation pathway • TiO2

1. Introduction

The occurrence of pharmaceuticals as environmental contaminants is reported for ground water, surface water as well as for sludge, soil and sediment samples [1]. Pharmaceuticals get into the environment from disposal of incompletely metabolized and unused drugs, solid waste landfills and wastes of animal feedlots. The wide range of pharmacological properties put hormonal corticosteroids, including prednisolone, among the most used drugs in the world [2]. These are able to affect aquatic organisms in the smallest concentrations [3]. Toxic effects of corticosteroids on aquatic organisms are exhibited as inhibition of locomotion, aggressive behaviour, immunological responses and altered reproductive cycles [1,4].

Widely present in the influents of the wastewater treatment plants, pharmaceuticals, including steroids, are often incompletely removed by the secondary wastewater and excess sludge treatment, including aerobic oxidation [1], and methane digestion of sediments [4]. As a result, frequent occurrence of pharmaceuticals in the aquatic environment as well as in the drinking water has raised a concern about their potential impact on environmental and public health. Various treatment technologies have been evaluated for pharmaceutical

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removal in recent years, including application of ozone and ozone/hydrogen peroxide, and membrane filtration [5]. Ozonation of pharmaceuticals in low concentrations, however, requires long treatment time and, thus, high doses of expensive ozone are needed doses of expensive ozone are needed [6]. Even more expensive membrane filtration creates retentate with elevated pollutant concentrations requiring further handling [7].

Concentrations of prednisolone present in natural aquifers are reported to be in ng L\(^{-1}\) scale [5], however, the content in sludges may reach up to 200 µg kg\(^{-1}\) [8]. In this study, higher concentrations of prednisolone were used in order to reliably determine its degradability by photocatalytic oxidation, and its degradation by-products. Regularities of prednisolone degradation at elevated concentrations could be extrapolated to lower concentrations, since photocatalytic oxidation has been shown to be kinetically controlled at lower pollutant concentrations [9]. Major physico-chemical properties of prednisolone are summarised in Table 1.

Photocatalytic oxidation is based on the action of positively charged holes on the illuminated surface of semiconductor, most often TiO\(_2\). Water molecules decompose on the holes forming hydroxyl radicals [15]; the hole, having even higher oxidation potential than the OH-radical [16], can also directly degrade the pollutants adsorbed on the surface of the photocatalyst, making adsorption often important in photocatalytic oxidation. The ratio of radical to hole oxidation depends on the adsorptive and reactive properties of pollutants [17-19], reflected also in the nature of oxidation products. However, titania photocatalysts can use only UV fraction (4%) of solar radiation due to the high band gap energy [20]. Hence, sensitising TiO\(_2\) to visible light enables to widen the utilised solar spectrum. The sensitisation could be achieved by doping TiO\(_2\) with various elements reducing the band-gap [21-23] for excitation of electrons by lower energy photons. However, the authors found earlier that the doped titania photocatalysts may oxidise only selected pollutants, such as methyl-tert-butyl ether, tert-butyl alcohol, i-propanol, p-toluidine, amoxicillin, but not phenol, humic substances, 2-ethoxy ethanol and ethylene glycol [9,24,25]. This can be explained by the smaller redox potential and, possibly, faster electron-hole recombination [26]. The reduced redox potential of new catalytic materials may still be high enough to produce hydroxyl radicals, although some substances may escape oxidation due to their susceptibility to hole oxidation only.

The authors failed to find any information in the scientific literature considering photocatalytic oxidation of corticosteroids, including prednisolone. The study on feasibility of prednisolone photocatalytic oxidation, also in presence of co-pollutants, with the elucidation of its oxidation pathways was one of the objectives of the present research. Another objective was characterisation and study of visible light active photocatalysts testing their performance towards the degradation of prednisolone. The applicability of doped photocatalysts under visible or solar irradiation as the pre-treatment stage before biological oxidation, i.e., the toxicity of the catalysts, not considered before was also studied.

### 2. Materials and methods

#### 2.1. Experimental setup and procedures

Two 200-mL thermostated (20±1°C) batch reactors with inner diameter of 100 mm, irradiated plane surface 40 m\(^2\) m\(^{-3}\), with magnetic stirrers, were used: the one used for the photocatalytic oxidation further designated as “active” and the other containing no photocatalyst further designated as “reference”, i.e., blank experiments, exposed to the identical conditions. The samples from both reactors were compared to avoid errors caused by water evaporation. A UV–light source, Philips Actinic BL low pressure luminescent mercury UV-lamp (15 W) with maximum emission around 365 nm was positioned horizontally over the reactors, providing the irradiance of 1.5 mW cm\(^{-2}\) at their surface, measured by the radiometer Micropulse MP100. The illuminance of the daylight fluorescent lamp (Philips TL-D 15W/33-640) was measured using TES 1332 luxmeter reaching 3,700 lx (l m\(^{-2}\)), which corresponds to the irradiance of 0.6 mW cm\(^{-2}\) [27]. For Philips TLD lamp, the amount of UV-irradiation was measured at 254 and 365 nm using the above mentioned Micropulse radiometer and total amount of UV was measured with Ocean Optics USB2000+UV-VIS-ES spectrometer. Only negligible fraction of UV around 365-400 nm was observed, which is in accordance with the data provided by the Philips TLD manufacturer. Thus, when using Philips TLD lamp,
any noticeable action of the photocatalyst is clearly due to the activity of the catalyst in visible light region. The prednisolone photocatalytic oxidation experiments were additionally conducted outdoors using natural solar radiation; the irradiance was measured by luxmeter approximating 16 mW cm$^{-2}$.

TiO$_2$ was used as a slurry of 1 g L$^{-1}$. The prednisolone (Sigma-Aldrich) concentration varied from 10 to 100 mg L$^{-1}$. The pH was adjusted with sulphuric acid (4 N) or sodium hydroxide (15%) and the impact of pH was studied in the range from 3 to 10. The pH changes were monitored during the runs, pH was not kept constant, and its changes were monitored. The treatment times chosen according to pre-screening results were 2 h under artificial irradiation, and 1 h under natural solar irradiation. Such treatment conditions were chosen in order to achieve at least 50% reduction of the initial prednisolone concentration, as complete conversion may require greatly increased treatment time, and will not give additional information on the possibility of prednisolone degradation by photocatalysis. Experiments were carried out three times under identical conditions with the average deviations under 5%.

Carbamide and sucrose, used as model co-pollutants in prednisolone photocatalytic oxidation experiment in order to simulate municipal wastewater, were used in the amounts of 75 and 50 mg L$^{-1}$, respectively. These concentrations broadly reflect normal values in domestic sewage [28] and are in agreement with previously conducted experiments [29]. These additives simulated wastewater with chemical oxygen demand (COD) of 145 mg O L$^{-1}$ and 35 mg L$^{-1}$ of total nitrogen.

tert-Butyl alcohol (TBA) as a radical scavenger was added to the treated prednisolone solution at molar ratio of [prednisolone]:[TBA] = 1:2 in order to determine the dominance of reactions taking place on titanium dioxide surface vs. reactions in the solution in the vicinity of the surface in prednisolone photocatalytic oxidation. These experiments were carried out at near-neutral pH with the prednisolone concentration of 10 mg L$^{-1}$.

Adsorption experiments were carried out in thermostated flasks with magnetic stirrers at 20±1°C. The amount of prednisolone adsorbed on titanium dioxide was derived from the mass balance by determining the concentration of the dissolved substance before adsorption and after the equilibrium was reached in 30 min, determined experimentally. With P25 titanium dioxide (Evonik), the adsorption experiments were performed in the prednisolone concentration range of 10 to 100 mg L$^{-1}$. With doped titania samples, adsorption of prednisolone was studied at 25 mg L$^{-1}$, in order to compare the photocatalysts’ adsorption capabilities.

### 2.2. Synthesis of photocatalysts

Specimens of carbon-containing titania were obtained by hydrolysing tetrabutyl orthotitanate at room temperature with unadjusted pH around 5.5 to 6.0, followed by thermal treatment in a range between 200 and 400°C. Three sulphur-containing photocatalysts used in this study were prepared by hydrolysing tetrabutyl orthotitanate with addition of a pre-calculated amount of 0.1 N sodium thiosulphate as a sulphur source, followed by calcination at 400°C. Three iron-containing titania catalysts with calculated iron content from 0.4 to 1.3 at.% were prepared by pulverisation of 75 mL of tetrabutyl orthotitanate into 1 L of pre-sonicated Fe$_2$O$_3$ suspensions of various concentrations (from 0.1 to 0.5 g L$^{-1}$). The hydrolysis was followed by sonication, drying and calcination at 200°C. After 4-h calcination, all the catalysts were washed with hot, 70 to 80°C, distilled water applied in a sequence of fifteen rinsing rounds, making about 1 L per 1 g of catalyst in total, to clean the catalyst from water-soluble compounds. In order to determine possible leaching of the dopants, the catalysts were dispersed in distilled water and subjected to UV-irradiation for overnight. After the catalyst separation, the possible content of dopants was analysed as follows: carbon species in the water were analysed for chemical oxygen demand (COD, see Section 2.3), the contents of nitrate and sulphate were analysed using Metrohm 761 Compact IC ion chromatograph; ammonia content was analysed photometrically by a modified version of standard phenate method [30]. The iron ions leakage was analysed using modified version of standard phenantroline method [30]. No leakage of any dopant was detected within the accuracy of the methods used.

### 2.3. Analytical procedures

High-performance liquid chromatography combined with diode array detector and mass-spectrometer, (HPLC-PDA-MS, Shimadzu LC-MS 2020) was used for the determination of prednisolone concentration. Phenomenex Gemini-NX 5u C18 110A 150×2.0 mm column, inner diameter 1.7 µm, was used with two eluents, 0.1% acetic acid aqueous solution (eluent A), and acetonitrile (eluent B), with total eluents flow of 0.3 mL min$^{-1}$. The starting concentration of eluent B was 5%, increased to 48.5% by 23 minutes with linear gradient, held at that concentration for two minutes, and then decreased to 9.5% by 30 minutes, out of 30 minute analysis. Mass spectra were acquired in full scan mode, MS operated in positive ionisation mode with interface voltage of 4.5 kV, and detector voltage of 3.3 kV. Diode array detector was set to scan samples at 190–800 nm. The instrument was operated and the results obtained with MS and PDA detectors were handled using
Shimadzu LabSolutions software. The by-products of prednisolone photocatalytic oxidation were determined using the described HPLC-MS method, at different initial pH values (3.0, 6.8 and 10.0).

Chemical oxygen demand (COD) was determined by a standard dichromate method [30], using HACH kit LCK 314 (15 to 150 mg O L⁻¹). This parameter was used to evaluate the oxidation degree of prednisolone, reflecting, unlike total organic carbon (TOC), not only the prednisolone mineralization, but also the changes in oxygen incorporation to organic matter during photocatalytic oxidation.

The crystallinity of carbon-containing titania was analysed using D5000 Kristalloflex, Siemens X-ray diffraction spectroscopy (XRD), with composition calculated by Topas R software, and sulphur- and iron-containing specimen were analysed with Scintag PAD V XRD (Cu Kα irradiation source in both cases). The specific surface area (SSA: BET and Langmuir adsorption) and the pore volume were measured by the adsorption of nitrogen using KELVIN 1042 sorptometer.

The isoelectric points (IEP) of pure and sulfur-, carbon and iron-doped titanium dioxide suspensions were determined by potentiometric titration using a Zetasizer Nano-ZS equipped with an MPT-2 autotitrator and vacuum degasser (Malvern Instruments, UK). The technique (Laser Doppler Micro-electrophoresis) is based on the electrophoretic light-scattering that measures migration rate of dispersed particles under the influence of an electric field. Zeta, ζ, potential was calculated by Malvern software using Smoluchowski equation. Suspenions of 1 g L⁻¹ TiO₂ (P25) and doped TiO₂ were prepared in deionized water, sonicated for 30 min (70 W, 42 kHz, Branson 1510, Branson Ultrasonic Corporation, USA) and diluted to 100 mg L⁻¹ in deionized water. Automatic titrations were performed in 10 ml as an initial sample volume, from pH 11 to pH 1.5 with 0.25 M NaOH and 0.25 M HCl. pH increment was 0.5 and target pH tolerance was 0.2. Each ζ-potential value recorded was the average of 10 measurements.

Protozoan culture (Tetrahymena thermophila strain BIllII) used for toxicity analysis was cultivated as described previously [31]. The cells were harvested during the exponential growth phase (cell density 5×10⁵ cells mL⁻¹) by centrifugation at 300 g for 5 min at 4°C and washed twice with MilliQ water. In order to evaluate cell density the cells were counted in haemocytometer (Neubauer Improved, bright line; Germany) after immobilising the cells in 5% formalin. To conduct the toxicity test 100 µL of the photocatalyst in MilliQ water was pipetted into the wells of 96-well polystyrene plates (Falcon) and 100 µL of T. thermophila cells in MilliQ water was added to the wells. The final photocatalysts concentrations in the test were 50, 500 and 1000 mg L⁻¹, each concentration was tested in three replicates and the final cell density in the test was 5×10⁵ cells mL⁻¹. The protozoan culture in MilliQ water and photocatalysts in MilliQ water were used as controls. The exposure of the protozoa to photocatalysts was performed comparatively under illumination and dark conditions for 2 and 24 h at 30°C with moderate shaking. The plates were illuminated from below using Philips TL-D 38 W aquarelle fluorescent tubes. The pH of T. thermophila control culture in MilliQ water was 6.8 and did not change significantly upon the addition of the photocatalysts. The pH remained unchanged during the 24-hour toxicity test. The viability of the cells was evaluated by measuring the ATP content using the luciferin-luciferase method as described previously [31] with slight modifications. The EC₅₀ (effective concentration leading to a 50% cell death) values were calculated from concentration-effect curves with REGTOX software for Microsoft Excel™ [32].

### 2.4. Photocatalytic oxidation efficiency

To express the results of prednisolone photocatalytic oxidation, the photocatalytic oxidation efficiency E, defined as the decrease in the pollutant amount divided by the amount of energy reaching the treated sample, was calculated according to Eq. 1 [33]:

$$E = \frac{\Delta C \times V \times 1000}{I \times s \times t}$$

where $E$ – photocatalytic oxidation efficiency, mg W⁻¹ h⁻¹; $\Delta C$ – the decrease in the pollutant’s concentration, mg L⁻¹; or COD, mg O L⁻¹; $V$ – the volume of the sample to be treated, L; $I$ – irradiance, mW cm⁻²; $s$ – irradiated area, cm²; $t$ – treatment time, h.

### 3. Results and discussion

#### 3.1. Dark adsorption experiments

The most efficient prednisolone (initial concentration 25 mg L⁻¹) adsorption on P25 was observed at near-neutral pH, followed by acidic media and being negligible in basic solutions as shown in Fig. 1a. This may be explained by charging of both TiO₂ surface and prednisolone molecules in acidic (minor positive charging of prednisolone) and alkaline (major negative charging of prednisolone) media, that results in electrostatic repulsion of prednisolone molecules in both cases; however, this is strongly pronounced only in alkaline media. Since no significant charging of TiO₂ occurs in neutral media, prednisolone adsorption improves. A similar trend was observed with all other catalysts studied.
The prednisolone dark adsorption on the P25 in neutral media ranged from 0.8 to 15 mg g\(^{-1}\) at the initial prednisolone concentration from 10 to 100 mg L\(^{-1}\) respectively. The adsorption experimental data fits well to the Freundlich equation (Eq. 2, \(R^2=0.9675\)):

\[
q_v = A \times C^m,
\]

where \(q\) – adsorption, mmol g\(^{-1}\); \(A\) and \(m\) – empirical constants, giving \(A = 0.262\) and \(m = 1.25\).

Fig. 1b shows the adsorption results for prednisolone in 25 mg L\(^{-1}\) solution on three synthesized catalysts, carbon-containing and iron- and sulphur-doped TiO\(_2\), compared to undoped P25 TiO\(_2\), as the decrease of prednisolone normalised adsorption at pH 6.7. The adsorption of prednisolone on the doped catalysts showed that over 10% of initial prednisolone was adsorbed on their surface at best, while up to 20% of prednisolone adsorbed on P25 from the solution with prednisolone initial concentration of 25 mg L\(^{-1}\). No prednisolone adsorption was detected on S-TiO\(_2\), indicating that increased specific surface area of the doped photocatalysts does not necessarily lead to an improved adsorption.

### 3.2. Photocatalytic oxidation experiments with P25 (Evonik)

Similarly to the adsorption pattern, the best photocatalytic oxidation performance was observed at pH 6.7, occurring naturally in the prednisolone solutions, followed closely by acidic media (pH 3), with the least effective performance observed in alkaline solutions (pH 10) (data not shown for the sake of brevity). Such pH dependence was earlier observed with e.g. alcohols [34-36] to which prednisolone is expected to exhibit similar behaviour due to its chemical structure. The decrease in the photocatalytic oxidation efficiency in alkaline media favourable for the reactions of hydroxyl radicals in the vicinity of photocatalyst surface indicates the minor role that mechanism in photocatalytic oxidation of prednisolone. Since photocatalytic oxidation efficiency under acidic and near-neutral conditions is higher, the prevalence of oxidation of adsorbed prednisolone on the photocatalyst surface, as opposed to previously mentioned mechanism, favoured by alkaline media, is assumed. This assumption finds confirmation in the prednisolone adsorption properties on P25 (see Section 3.1). However, the prevalence of oxidation of molecules adsorbed on the photocatalyst surface may concern only the starting point of prednisolone oxidation. The prednisolone molecule that has lost one or more electrons on titanium dioxide surface may subsequently react both there and with hydroxyl radicals in the solution in the vicinity of the photocatalyst surface.

The efficiency of COD reduction has the same trend as efficiency calculated for prednisolone concentration reduction. However, it yields to the efficiency of prednisolone removal demonstrating slower degradation rate of prednisolone photocatalytic oxidation by-products. The significant difference, for almost 40%, in the efficiency of COD reduction between neutral and acidic media indicates lower reactivity of prednisolone photocatalytic oxidation by-products at lower pH. Thus, it could be assumed that since neutral medium is the most favourable for oxidation of prednisolone by-products, photocatalytic oxidation of by-products necessitates utilizing both mechanisms, while low efficiency in alkaline medium points that adsorption remains the prerequisite of photocatalytic oxidation.

Fig. 2 shows the dependence of photocatalytic oxidation efficiency \(E\) on the prednisolone initial concentration: \(E\) increases with the increased initial...
prednisolone concentration assuming the description of the photocatalytic oxidation using Langmuir-Hinshelwood (L-H) model of monomolecular surface reaction, followed by the products desorption (Eq. 3).

The equation, derived from the experimental data via the $1/r_0 = f (1/c_0)$ dependence (plot not shown), gives $k = 0.0044$ mM$^{-1}$ min$^{-1}$ and $K = 11.4$ mM at average square deviation $R^2 = 0.9999$, supporting the proposed L-H data fit:

$$r_0 = k \frac{Kc_0}{1 + Kc_0},$$

where $r_0$ is the reaction rate, mM min$^{-1}$; $k$ – reaction rate constant, mM$^{-1}$ min$^{-1}$; $K$ – adsorption constant, mM; $c_0$ – initial prednisolone concentration, mM.

The similar efficiencies of prednisolone and COD degradation at neutral pH implies a high degree of mineralization: indeed, at prednisolone concentrations up to 25 mg L$^{-1}$, prednisolone removal was around 90-96% with simultaneous COD decrease over 80% in 2 h of treatment time. At prednisolone concentration of 100 mg L$^{-1}$, the target pollutant and COD removal rates decreased to 57 and 30%, respectively, although the absolute oxidation rates increased. Thus, photocatalytic oxidation is able to degrade both target compound and its degradation by-products reasonably well.

### 3.3. Prednisolone photocatalytic oxidation reaction pathway

A number of organic by-products were determined qualitatively using HPLC coupled with Electrospray Ionization MS (ESI-MS, see Materials and Methods for more detail). The determined products allow to distinguishing four possible prednisolone photocatalytic oxidation pathways (Fig. 3), depending on initial pH of the treated solution. Two of them (A at pH3 and B at pH of 6.8 and 10) begin with stepwise oxidation of quinonic ring before finally forming the identified products with $m/z = 373$ and 371, respectively; from both the product with $m/z = 359$ is formed. Subsequently, in pathway A the hydroxymethylene group connected to the cyclohexanic ring (leftmost in the scheme) is oxidised stepwise, yielding products with $m/z = 357$ and 327. In pathway B, the abovementioned cyclohexanic ring undergoes direct cleavage, leading to the formation of product with $m/z = 345$. Pathway C, present at natural pH of prednisolone, begins with the oxidation of cyclopentanic part of the molecule, leading to the formation of products with $m/z = 323$ and 277, depending on whether the cyclohexanic part of the molecule adjacent to cyclopentanic is cleaved or remains intact; subsequent oxidation of both fragments leads to the formation of product with $m/z = 253$. In alkaline media, product with $m/z = 281$ was identified, suggesting a reaction pathway similar to C. Finally, pathway D, occurring at both natural and alkaline pH shows simultaneous oxidation of prednisolone molecule from both sides. The relative abundance of the reaction pathways is explained by the different prednisolone adsorption mechanisms taking place simultaneously: depending on which part of the prednisolone molecule is adsorbed first (i.e., quinonic cycle, or one of hydroxyl groups), the photocatalytic oxidation follows one of the oxidation pathways proposed. At acidic pH prednisolone, being a weak base as an alcohol, may be slightly protonated and thus positively charged, leading to electrostatic repulsion from the likewise positively charged titanium dioxide surface, whereas the quinonic group oxygen should be less affected by this, resulting in the domination of pathway A, which originates from prednisolone adsorption via quinonic group. At natural pH of prednisolone and at alkaline pH, electrostatic
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effects appear to be far less pronounced, resulting in a greater variety of oxidation products forming pathways B to D.

3.4. The influence of admixtures on prednisolone photocatalytic oxidation

The addition of tert-butyl alcohol did not exhibit any influence on the efficiency of prednisolone oxidation. This supports the hypothesis of the minor role of radical reactions taking place in the vicinity of the photocatalyst surface played in prednisolone photocatalytic oxidation when compared to oxidation of adsorbed prednisolone molecules on the surface of TiO₂.

The addition of carbamide showed no negative influence on the photocatalytic oxidation of prednisolone (see Fig. 4). This can be explained by the insignificant carbamide adsorption on the surface of titanium dioxide [29], that, in turn, means practically unhindered prednisolone adsorption, leaving overall photocatalytic oxidation performance largely unaffected. No noticeable carbamide oxidation was observed during the course of the experiments.

The addition of sucrose hindered the photocatalytic oxidation of prednisolone noticeably: around 20% of the initial concentration remained unoxidized, as opposed to complete prednisolone elimination without sucrose under identical experimental conditions (Fig. 4). This can be explained by the competitive adsorption of sucrose and prednisolone on the catalyst surface: adsorbed sucrose molecules hinder prednisolone adsorption, leading to a decrease in prednisolone photocatalytic oxidation rate. When carbamide and sucrose were added to prednisolone solution simultaneously, the decrease of prednisolone degradation rate was identical to that observed upon sucrose admixture (Fig. 4), with no synergism clearly observed.

These results show the ability of prednisolone to successfully undergo oxidation upon the addition of wastewater constituents, establishing the practicability of photocatalysis against corticosteroids.

3.5. Characterization of photocatalytic materials

Table 2 shows the characteristics of carbon-containing photocatalysts varying with calcination temperature. Within the calcination temperature range applied, the catalysts were composed of mainly anatase (>70%) and brookite (> 20%). X-ray diffraction curves for C-TiO₂ specimen can be seen in Fig. 5a; for anatase, (1 0 1), (0 0 4) and (2 0 0) peaks are the most pronounced, with (1 0 1) being the most distinctive, and for brookite, (1 2 0), (1 2 1), and (2 3 1) peaks were identified. Although no systematic changes in the catalysts’ phase

Figure 3. Proposed prednisolone photocatalytic oxidation reaction pathway.
composition were observed, specific surface area (SSA) clearly decreased with the increased C-TiO$_2$ calcination temperature from 200 to 400°C, whereas micropore area and volume increased. However, the previous results show that the surface area and pore volume of the modified titania photocatalysts do not always correlate with the adsorbed pollutants amount and the photocatalytic oxidation performance [9,24]. Thus, the surface data yield by their importance to the adsorption and photocatalytic oxidation efficiency [9].

Table 3 shows the composition and surface properties of S-TiO$_2$ photocatalysts after calcinations at 400°C. Judging from the XRD analysis (Fig. 5b), anatase is the main crystallographic form, with (1 0 1), (0 0 4), (2 0 0), (1 0 5), (2 1 1) and (2 0 4) peaks identified; (1 0 1) peak has the highest intensity. Sulphur-containing catalysts are described in more detail in previous paper from the authors [25].

The calculated amount of iron in the Fe-TiO$_2$ catalysts calcinated at 400°C was 0.4, 0.9 and 1.3 at.%. XRD data for iron-containing titanium dioxide specimen used in this study is provided in Fig. 5c. All specimen exhibited (1 0 1), (0 0 4), (2 0 0), (1 0 5) and (2 0 4) anatase peaks, with (1 0 1) being the most intensive here as well. It can be seen that at 0.4 at.% Fe the catalyst is pure anatase, whereas two other specimen with higher iron content show two well-pronounced α-Fe$_2$O$_3$ peaks – (1 0 4) and (1 1 0), with their relative intensity increasing with increasing iron content. This suggests that at elevated dopant concentrations a portion of iron remains unincorporated into titanium dioxide crystal lattice in these catalysts, as no iron species-associated peaks were observed at 0.4 at.% Fe, where at least partial incorporation is supposed lowering the measurable iron quantities to undetectable limits.
Figure 5. X-ray diffraction curves for carbon- (a), sulphur- (b) and iron-containing titanium dioxide (c); A – anatase, B - brookite.
Potentiometric titration curves of 100 mg L\(^{-1}\) P25, S-TiO\(_2\) (0.8 at.%) and Fe-TiO\(_2\) (0.4 at.%) suspensions are presented in Fig. 6. The doping agents decreased the values of isoelectric point of titanium dioxide suspension: C-TiO\(_2\) IEP = 3.69 ± 0.12, S-TiO\(_2\) IEP = 3.59 ± 0.04 and Fe-TiO\(_2\) IEP = 2.90 ± 0.12; for comparison, IEP of P25 titanium dioxide has been reported to be around 6 [37].

It has been shown previously that P25 was harmful (EC\(_{50}\) < 100 mg L\(^{-1}\)) to the "young" and "old" algal cultures [38] and phototoxic to bacteria: concentration of 100 mg TiO\(_2\) L\(^{-1}\) was lethal to 75% of the *Escherichia coli* population whereas the same concentration of TiO\(_2\) in the dark showed no impact [39]. Thus, biological effects of applied photocatalysts on microorganisms present in the activated sludge process should be considered. Beside bacteria, ciliates constitute the second most relevant and abundant community in the activated sludge [40]. *Tetrahymena* are freshwater ciliate protozoa that have been used as model organisms for environmental research for years [41]. Furthermore, *Tetrahymena thermophila* is also present in the activated sludge process [42]. The biological effects of P25 and synthesized TiO\(_2\) photocatalysts on *Tetrahymena thermophila* were evaluated and average calculated EC\(_{50}\) values are presented in Table 4.

The results indicate that according to the risk phrases for ranking toxicity of chemicals for aquatic organisms (EC Directive 93/67/EEC) the tested photocatalysts could be classified as not harmful to *Tetrahymena thermophila* as all the EC\(_{50}\) values were above 100 mg L\(^{-1}\). Moreover, the EC\(_{50}\) values of the doped titania photocatalysts exceeded even 1 g L\(^{-1}\) which is the concentration used in the experiments of photocatalytic degradation of prednisolone. However, the illumination at VIS-range rendered P25 TiO\(_2\) about two times more toxic compared to the dark conditions. Additionally, compared to P25, doped TiO\(_2\) samples had milder effects on *T. thermophila* viability, indicating the lower redox potential of synthesized catalytic materials evidenced later in the course of photocatalytic oxidation of prednisolone (see Section 3.6).

3.6. Photocatalytic oxidation experiments with doped titania

During the course of the experiments the dopants were not washed out of the catalysts, indicate by the sustaining of their catalytic activity in sequential reuse and leaching tests (see Section 2.2). It can be thus clearly stated that the dopants were stably incorporated into the catalysts’ crystal lattice.

Negligible quantity of UV emitted by the VIS lamps (see Section 2.1) cannot explain the photocatalytic oxidation performance of the doped catalysts, as it does not correlate with the level of observed photocatalytic efficiency as compared to the results with the UV-irradiated P25. This observation indicates that visible light is responsible for photocatalytic oxidation due to dopant species. The performance of three doped titania catalysts containing carbon, sulphur and iron was tested with prednisolone solutions of 25 mg L\(^{-1}\) at pH 6.7 under VIS radiation (Fig. 7).

The S-TiO\(_2\) sample with sulphur content of 0.8 at.% showed the best photocatalytic oxidation performance under VIS irradiation reaching 67% of the efficiency of UV-radiated P25 photocatalyst. Iron-doped and carbon-containing TiO\(_2\) had relatively low efficiency. The efficiency of iron-doped TiO\(_2\) increased slightly with iron content achieving the highest value at the iron content 0.3 at.%; nevertheless, the best iron-doped TiO\(_2\) was about three times lower in efficiency than the P25 under UV-light. Similarly, the performance of carbon-containing TiO\(_2\) under VIS light increased slightly with
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The decrease in catalysts’ calcination temperature, the latter is expected to result in higher carbon content.

The differences in behaviour of visible light-sensitive photocatalysts containing different dopants may be explained by different nature of the dopants and the corresponding changes in the catalysts’ band gap and redox potential. The comparison of the photocatalytic oxidation and adsorption results obtained with the doped catalysts show a consistence of both photocatalytic oxidation efficiency and adsorption for iron-doped and carbon-containing catalysts. The reason for poor adsorption of prednisolone at S-TiO$_2$ despite its surface area exceeding the one of P25 remains unknown, especially taking into account the overall similarity of the catalysts’ IEP and zeta-potential values at the pH of the experiments. However, sulphur-doped catalysts show the highest photocatalytic oxidation efficiency at no adsorption observed (see Section 3.2). Unlike carbon and iron-containing photocatalysts, S-TiO$_2$ exhibited strongly acidic properties: solution pH decreased once the catalyst was added to water. Since the catalysts were thoroughly washed upon preparation, and no sulphates leaching was detected, the acidic reaction should originate from partially bonded sulphate on the catalyst surface and resulting in negative catalyst surface charge due to the sulphates’ dissociation. On the other hand, in such conditions the prednisolone molecule may be expected to act as a weak base having its hydroxyl groups partially protonated and the prednisolone molecule or its part charged positively. As a result, the prednisolone molecule may be drawn into close vicinity of the catalyst surface, where it can react with the hydroxyl radicals. Though prednisolone is better adsorbed onto the surface of C- and Fe-TiO$_2$, the photocatalytic oxidation efficiency of these catalysts is low. The latter is in agreement with the data reported by Liu et al. [26], indicating the redox potential of doped catalysts’ holes being lower than that of undoped titania, consequently resulting in reduced photocatalytic oxidation rate.

### 3.7. Solar photocatalytic oxidation experiments

The performance of prednisolone photocatalytic oxidation under solar irradiation was studied with P25 industrial photocatalyst and with sulphur-doped (0.8 at.%) photocatalyst, utilizing the VIS portion of solar...
light along with UV. The main goal of these experiments was not only convincing that the photocatalysts applied can successfully operate under natural irradiation, but mainly to investigate the changes in their performance under solar light. The results obtained under solar radiation were compared to the ones achieved under artificial UV and VIS (see Section 2.1) (Fig. 8).

Degradation of prednisolone with P25 and sulphur-doped TiO₂ under solar radiation was expectedly faster than under artificial UV- or VIS-light. However, the improvement of the photocatalytic oxidation rate observed for different catalysts was also different. For example, prednisolone was degraded with P25 for 94% and 73% in 60 min under solar irradiation and artificial UV, respectively. With S-TiO₂, the corresponding numbers were 58% and 15%. Thus the performance of the sulphur-doped catalyst increased to a greater extent than that of P25, even though the latter was still more effective. The reason behind the different response of the catalysts to natural solar radiation lays in the higher intensity of ultraviolet and visible radiation in solar spectrum (see Materials and Methods section).

4. Conclusions

Aqueous photocatalytic oxidation of prednisolone was studied using P25 (Evonik) and visible lightsensitive synthetic sol-gel carbon-containing titania catalysts used as is or doped with sulphur and iron. Photocatalytic oxidation was shown to be an effective method for prednisolone degradation with near-complete prednisolone removal at lower concentrations, and the observed oxidation efficiency up to 40 mg W⁻¹ h⁻¹. A number of prednisolone photocatalytic oxidation by-products determined in the present study allowed the suggestion of possible reaction pathways proceeding simultaneously: the stepwise oxidation of the terminal parts of the prednisolone molecule, quinonic ring or cyclopentanic part, or oxidation from both quinonic and cyclopentanic part at the same time was assumed.

In the presence of major wastewater constituents, carbamide and sucrose, prednisolone photocatalytic oxidation also proceeded well with a minor disturbance from sucrose. The best prednisolone photocatalytic oxidation results were achieved at near-neutral pH, natural to prednisolone solutions.

The efficiency of doped titania of up to 13 mg W⁻¹ h⁻¹ under visible light was inferior to the one observed with P25 under UV; higher efficiencies were observed as expected for the solar light application. Sulphur-doped titania (0.8 at.%) showed the best photocatalytic oxidation performance among the doped photocatalysts tested although yielding to the commercial P25 under both artificial and solar radiation conditions. The nontoxicity of doped catalysts towards Tetrahymena thermophila, a ciliate protozoa present in the activated sludge, indicates their lower oxidative ability, but also implies their potential application in pretreatment of toxic hazardous materials under VIS or solar radiation before the biological degradation stage.

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