Analytical Methods

Roxithromycin degradation by acidic hydrolysis and photocatalysis

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Roxithromycin degradation by acidic hydrolysis and photocatalysis

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Abstract

The purpose of this work was to study two methods of degradation of roxithromycin; acidic hydrolysis and photocatalytic degradation under illumination with near-UV light in the presence of the TiO_2 photosensitizer. For acidic degradation studies a TLC-densitometric method have been developed using TLC aluminium plates precoated with silica gel F_{254} and the mobile phase composed of methanol-acetone-ammonia 25% (1:14:0.1, v/v/v) which gives compact spots for roxithromycin and its degradation products. Photocatalytic degradation studies in the presence of TiO_2 were monitored by HPLC. The obtained results have shown that both methods of degradation of roxithromycin are quite efficient. Kinetics of first order photocatalytic degradation reaction follows the Langmuir-Hinshelwood theory for photocatalytic microheterogeneous systems. The chemical structures of the degradation products obtained during acidic degradation and photocatalytic degradation were proposed based on UPLC-MS/MS technique. The general mechanisms of both processes were proposed.

Key words: roxithromycin, degradation studies, kinetic evaluation, TiO_2 photosensitizer, quantitative determination, photocatalysis

Introduction

Pharmaceuticals are a class of substances which raise increasing environmental concern due to their strong bioactivity [1], on one hand, and their growing concentration and widespread distribution in the environment, on the other [2]. This is, at least in part, due to the fact that the pharmaceuticals present in the hospital wastewater are almost always introduced untreated to the municipal sewage system and ultimately to the municipal wastewater treatment plants (WWTPs). The most potent and harmful pharmaceuticals in the hospital wastewater include antibiotics, hormones, enzymes, MRI and X-ray contrast agents, anesthetics, psychopharmaceuticals, disinfectants and cytostatics [3-5]. Among these substances, antibiotics are of special interest because they cause antibiotic resistance of (pathogenic) bacteria that is one of the most important challenges of the modern medicine worldwide [6].

Macrolides (MLs) such as erythromycin, clarithromycin, roxithromycin, and azithromycin, are the second most important class of antibiotics used in human therapy after the β-lactams [7]. Roxithromycin (Roxy), ((E)-erythromycin-9-[O-[(2-methoxyethoxy)-methyl]oxime]) is a second generation semi-synthetic macrolide antibiotic derivative of erythromycin A (Figure 1). It exists in an E-configuration [8]. This molecule is composed of a 14-membered lactone ring, a desosamine sugar unit and a cladinose sugar unit. A significant difference between the structures of both macrolides lies at position 9 of the lactone ring, where Roxy has an etheroxime chain instead of a carbonyl group at the same position in erythromycin A.
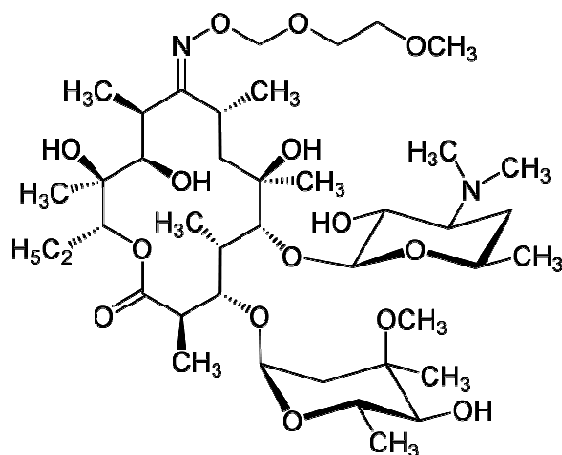
Figure 1. Chemical structure of roxithromycin

Roxy is the least active of the 14-membered macrolides having activity against gram-positive and gram-negative cocci, gram-positive bacilli and some gram-negative bacilli [9,10].

Roxy has been frequently detected worldwide in the effluents of WWTPs [11,12], surface waters [13], and even a drinking water [14]. Due to the widespread use of Roxy it is important to find out its metabolites and the most efficient methods of its degradation before it reaches the environment. Oxidative dealkylation of the alkylether side chain, hydrolytic cleavage of the sugar cladinose, and O-demethylation at the oxime side chain are the major metabolic pathways of Roxy in humans [8]. Metabolic isomerization of the oxime is stereoselective leading to the formation of Z-oxime isomers [15,16].

The acidic hydrolysis of Roxy in simulated gastric fluid (SGF) has been already reported [17]. Moreover, the degradation of clarithromycin and Roxy adsorbed on to iron(III) and manganese(IV) oxides was studied [7]. It was found that the degradation of these MLs proceeds both by the hydrolysis of the cladinose sugar and the lactone ring, with the N-dealkylation of the amino sugar being the minor pathway. Sorption and transformation of MLs on clays have been also described [18]. Biodegradation studies of Roxy in activated sludge process have shown that hydrolysis of Roxy is negligible and the antibiotic removal proceeds mainly through adsorption [19].

The aim of this work was to study the degradation of Roxy by acidic hydrolysis and sensitized photodegradation as possible methods of its treatment before it is transferred into the environment.   We have performed a detailed analysis of the degradation pathway under these conditions. We also propose the alternative degradation method, a photocatalytic degradation of Roxy using TiO_2 dispersion. To the best of our knowledge no papers presenting UV-light induced photocatalytic degradation of Roxy sensitized by suspended TiO_2 are available in the literature, although systems containing TiO_2 coated on carriers such as cenospheres have been reported [20].

Experimental

Apparatus

The degraded Roxy samples were analyzed using UPLC-MS/MS system - ACQUITY UPLC and TQD mass spectrometer (Waters Corporation, Milford, MA, USA) and a HPLC system composed of a Waters 515 HPLC pump, a 2996 Photodiode Array Detector, and a 717 Plus Autosampler (Waters Corporation, Milford, MA, USA). TLC analyses were performed using a TLC Scanner 3 densitometer with Cats 4 software (Camag, Muttenz, Switzerland). Irradiations were performed using a Rayonet RPR-100 photoreactor (Southern New England Ultraviolet Company, Brandford, CT, USA). Samples were dried using a drying oven with natural convection – Binder ED 115/E2 (Binder GmbH, Tuttlingen, Germany) and a muffle furnace FCF 5S HM (Czylok, Jastrzębie Zdrój, Poland).

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Dynamic light scattering

Dynamic light scattering (DLS) of TiO_2 dispersed in water was used to estimate the size of the average TiO_2 particle. The measurement was carried out using Malvern Zetasizer Nano-ZS. Anatase refractive index of 2.55 was used in the calculations.

UV-Vis reflectance spectroscopy

Reflectance spectrum of the TiO_2 powder was acquired using an Ocean Optics USB2G5726 spectrophotometer equipped with an AvaLight-DHS lamp and an integration sphere. The bandgap energy was estimated using Tauc plot.

Powder X-Ray diffraction (PXRD)

The PXRD analyses were carried out to assess the crystal structure of the obtained TiO_2. The measurement was carried out using X'PERT PRO diffractometer. Scherrer equation was used to approximate the crystallite size:

$$L = \frac{K\lambda}{\beta_{1/2}\cos\theta}$$

where:

L – average crystallite size, λ – the wavelength of X-ray radiation, K – shape constant equal to 0.9 for spherical particles, $\beta_{1/2}$ – the line broadening at half the maximum intensity, θ - Bragg angle

Materials and reagents

Roxithromycin - (3R,4S,5S,6R,7R,9R,11S,12R,13S,14R) - 6 - {[(2S,3R,4S,6R) - 4-(dimethylamino) - 3 - hydroxy - 6 - methyloxan – 2 - yl]oxy} - 14 - ethyl - 7,12,13 - trihydroxy - 4 - {[(2R,4R,5S,6S) - 5 - hydroxy - 4 - methoxy - 4,6 - dimethyloxan - 2 -yl]oxy}

- 3,5,7,9,11,13 - hexamethyl - 10 - (2,4,7 - trioxa - 1 - azaoctan - 1 - ylidene) - 1 -
oxacyclotetradecan-2-one was purchased from LGC Standards, EPR 1500000, Teddington,
London, England). The following commercial Roxy preparations were used: Rulid 150 mg
(Sanofi-Aventis, Paris, France), Xitrocin 150 mg (Polfa Pabianice, Pabianice, Poland),
Rolicyn 150 mg (Polfa Tarchomin, Warsaw, Poland), Renicin 150 mg (Sandoz, Kundl,
Austria). Ethanol (POCh), methanol (Merck), acetone (Chempur), ammonia 25% (POCh),
sulfuric acid 95% (POCh), hydrochloric acid (POCh), water (Merck), acetonitrile (Merck),
formic acid (Merck), titanium (IV) isopropoxide 97% (Sigma-Aldrich), KH_2PO_4 (POCh) were
of analytical or HPLC purity and used as received.

Standard solutions

Standard solutions were prepared by dissolving an appropriate amount of the standard
substance of Roxy in ethanol to give a concentration of 0.025% (w/v) for linearity and
precision studies and 0.0025% (w/v) for the determination of LOD and LOQ. Standard
solution of Roxy at a concentration of 0.6 % (w/v) was prepared for the degradation studies in
acidic solutions.

TLC-densitometric conditions

Standard solution of Roxy was applied in 8 mm bands, 10 mm apart, and 10 mm from
the lower edge to TLC silica gel 60 F_{254} plates (10×15 cm) using Camag sample applicator
Linomat 4 equipped with a 100 μL syringe. Chromatograms were developed over a distance
of 145 mm using methanol-acetone-ammonia 25% (1:14:0.1, v/v/v) in a chromatographic
chamber saturated for 10 min with mobile phase vapor at room temperature. The mobile
phase was chosen experimentally after checking 20 different mobile phases. After
development, the TLC plates were dried at room temperature and spots detected with freshly

prepared concentrated sulfuric acid-ethanol (1:4, v/v) mixture. After drying the plates were sprayed with the reagent, then heated in an oven at 100°C for 5 min. After spot detection, the plates were scanned in the visible range at 483 nm, which was chosen on the basis of an absorption spectrum recorded directly from the chromatogram. The analytical wavelength was the same for Roxy and its degradation products. Densitometric scanning was carried out using a Camag TLC Scanner 3 equipped with a tungsten lamp in the linear absorbance-reflectance mode controlled by Cats 4 software resident in the system. The slit dimensions were 8×0.6 mm, the scanning speed was 20 mm/s, and data resolution was 100 μm/step. Retardation factors (R_F values) were used to identify Roxy and its possible degradation products. For quantitative determination of Roxy in the degradation studies, the scan areas of appropriate peaks were recorded and the relative percent concentrations computed by employing the internal normalization method, according to the formula: $i [\%]=(x_i/\Sigma x) 100$, where $i [\%]$ is constituent concentration, x_i is peak area for the determined constituent, and Σx is sum of peak scan areas in the chromatogram.

Determination of Roxy in commercial pharmaceutical preparations

Three μL of the Roxy standard solution and the analyzed solutions at a concentration of 0.025% (w/v) were applied to the chromatographic plates with a Linomat in the form of a band 8 mm in width. Chromatograms were developed under the conditions described in the TLC conditions section. The content of antibiotics in drugs was computed by comparing the peak areas for standard and tested solutions. The results of determination for preparations under investigation are listed in **Table S2** (see Supplementary Information).

Acid-induced hydrolysis of Roxy

Hydrolysis of Roxy was carried out at pH 1.30, 1.70, and 2.30 at 40, 50 and 60°C. In

a 8 mL borosilicate glass vial 2 mL of 0.6% (w/v) Roxy standard solution was mixed with 2 mL of appropriate acid (0.1, 0.04 and 0.01 mol/L HCl) and heated in an oven to the desired temperature. Then 500 μL of incubated solutions were taken for the analysis in time intervals given in **Figure 2.** After cooling the solutions were mixed with ethanol at a 1:1 ratio. 10 μL aliquots of resultant solutions were applied to the TLC plates in triplicate. The TLC-densitometric method was carried out according to the developed method. For quantitative determination purposes, the surface areas of appropriate peaks were recorded, and the percentage concentrations of Roxy were computed by using internal normalization method. Presented results are the mean value of three measurements.

Kinetic testing

To characterize the degradation processes of Roxy, based on the relationship log(c)=f(t), where c is concentration and t stands for time, the order of degradation reaction was determined; then the rate constants k and $t_{0.1}$, $t_{0.5}$, E_a, and ΔH^{++} were calculated [21].

Photocatalytic degradation studies of Roxy in the presence of TiO$_2$

Synthesis of TiO$_2$

2 mL of titanium (IV) isopropoxide 97% was added to 10 mL of ethanol. Then the mixture of 2 mL of water in 10 mL of ethanol was added in 0.1 mL portions while stirring vigorously. The mixture was left on magnetic stirrer for 2 h and then 30 mL of water was added and mixture was centrifuged. The obtained solid was washed with water and dried in an oven at 60°C for 12 h. The obtained dry powder was heated in an oven for 2 h at 80°C and then for 2 h at 500°C. The dry product was triturated in an agate mortar.

Photocatalytic degradation studies of Roxy

5 mg of Roxy was dissolved in 50 mL of 0.01 M HCl to obtain 0.1 mg/mL solution of pH = 2 (HCl was used to increase solubility of Roxy). 25 mg of synthesized TiO_2 was introduced to 20 mL of the solution. The obtained dispersion was placed on a magnetic stirrer and was intensively bubbled with oxygen for 30 min. Then the mixture was irradiated for 30 min in the Rayonet reactor equipped with 6 lamps (8 W) at λ=350 nm. 4 mL samples were taken for analysis and the mixture was constantly bubbled with oxygen during irradiation. In order to separate TiO_2 from Roxy solution the samples were centrifuged for 10 min at 18000 rpm. Concentration of Roxy was monitored by HPLC method, using as the eluent methanol:0.03M KH_2PO_4 (50:50) mixture at a flow rate of 0.7 mL/min and a Biobasic-8 (250mm×4 mm, particle size 5 μm) Thermo Scientific column. To find out if the adsorption processes of Roxy on the surface of the photosensitizer occur the same experiment was conducted in the dark. To find out if the process of direct photolysis of Roxy takes place the same experiment was conducted in the absence of the photosensitizer.

Studies of the degradation products using UPLC-MS/MS

The UPLC-MS/MS system consisted of a Waters ACQUITY® UPLC® (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were carried out using the Acquity UPLC BEH (bridged ethyl hybrid) C18 column; 2.1 × 100 mm, and 1.7 µm particle size. The column was maintained at 40°C, and eluted under gradient conditions from 95% to 0% of eluent A over 10 min, at a flow rate of 0.3 mL min^{-1}. Eluent A: formic acid/water (0.1%, v/v); eluent B: formic acid/acetonitrile (0.1%, v/v). Chromatograms were recorded using Waters eλ PDA detector. Spectra were analyzed in 200 – 700 nm range with 1.2 nm resolution and sampling rate of 20 points/s. MS detection settings of Waters TQD

mass spectrometer were as follows: source temperature 150 °C, desolvation temperature 350°C, desolvation gas flow rate 600 L h^{-1}, cone gas flow 100 L h^{-1}, capillary potential 3.00 kV and cone potential 20 V. Nitrogen was used as both nebulizing and drying gas. The data were obtained in a scan mode ranging from 50 to 1000 m/z at 0.5 s intervals. Collision activated dissociation (CAD) analyses were carried out with the energy of 40 eV, and all the fragmentations were observed in the source. Consequently, the ion spectra were obtained by scanning from 50 to 1000 m/z range. Data acquisition software was MassLynx V 4.1 (Waters).

Results and discussion

The aim of this paper was to study the degradation of Roxy in acidic solutions and its photocatalytic degradation process in UV light in the presence of the TiO_2 photosensitizer. For both methods the degradation products of Roxy were identified.

Acidic hydrolysis of roxithromycin

Acid-induced hydrolysis of Roxy was carried out by the developed chromatographic-densitometric method. Using TLC F_{254} silica gel plates as a stationary phase and methanol-acetone-ammonia 25% (1:14:0.1, v/v/v) as the mobile phase well-developed and resolved peaks of Roxy and its degradation products were obtained. The spots were visualized with sulphuric acid in ethanol and scanned densitometrically at 483 nm. The method was validated according to ICH requirements (see Supplementary information, Method validation, Table S1).

Acidic hydrolysis of Roxy was performed at three different temperatures, i.e., at 40, 50, and 60°C, and for three different pH of 2.30, 1.70, and 1.30 (**Figure 2**). The relationship between Roxy concentration and time follows the kinetics of a first order reaction, consistent

with the results obtained for hydrolysis carried out in SGF [17]. To obtain full characteristics

of the degradation process, kinetic parameters such as k, $t_{0.1}$, and $t_{0.5}$ values describing Roxy

acidic degradation rate under different conditions were calculated together with the

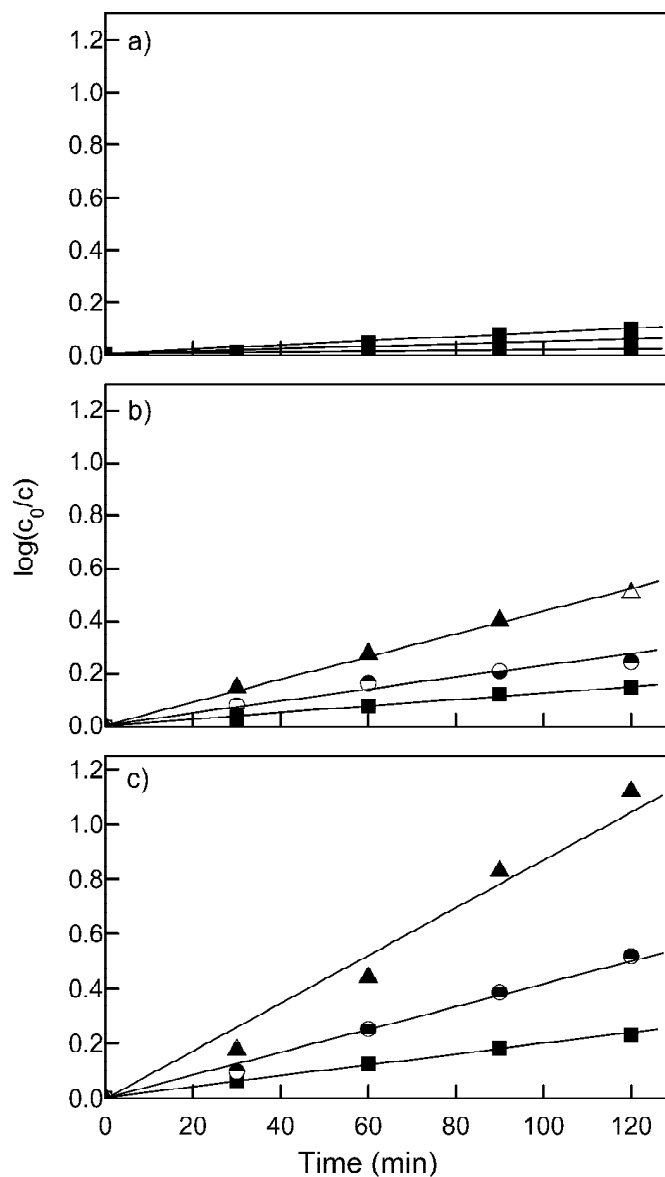thermodynamic parameters such as E_a and ΔH^{++} (**Table 1**).

Figure 2. The kinetic data for the acidic hydrolysis of Roxy for pH of a) 2.30, b) 1.70, and c) 1.30 at (■) 40, (●) 50, and (▲) 60°C.

Table 1. Kinetic and thermodynamic parameters calculated for Roxy acidic hydrolysis.

pH		Temperature (°C)			E_a (kJ/mol)	ΔH^{++} (kJ/mol)
		40°C	50°C	60°C		
2.30	k (h^{-1})	0.02	0.08	0.12	77.64	74.95
	$t_{0.5}$ (h)	38.45	8.75	5.99		
	$t_{0.1}$ (h)	5.84	1.33	0.91		
1.70	k (h^{-1})	0.17	0.29	0.59	53.92	51.23
	$t_{0.5}$ (h)	4.06	2.43	1.18		
	$t_{0.1}$ (h)	0.62	0.37	0.18		
1.30	k (h^{-1})	0.27	0.60	1.29	67.76	65.08
	$t_{0.5}$ (h)	2.59	1.16	0.54		
	$t_{0.1}$ (h)	0.39	0.18	0.08		

Note: k-reaction rate constant, $t_{0.5}$-half-life time, $t_{0.1}$-time in which concentration of Roxy is reduced by 10%, E_a-activation energy, ΔH^{++}-activation enthalpy.

Increase in both pH and temperature increases the rate of Roxy hydrolysis.

TLC analysis of the acidic solutions revealed two degradation products with R_F values of 0.82 and 0.87 besides the TLC peak of Roxy with R_F=0.42 (**Figure 3**). The obtained degradation products were subjected to UPLC-MS/MS analysis in order to propose their molecular weights and structures. The identification of the degradation products of E-Roxy induced by acidic conditions was performed on a basis of UPLC/MS analysis and supported with fragmentation patterns obtained from MS/MS experiments. The possible degradation products are shown in **Table 2**. The acidic degradation was found to affect mainly the L-cladinose substituent, causing its detachment from macrolide core, and to bring about isomerization of the oxime moiety from the native form E to Z.
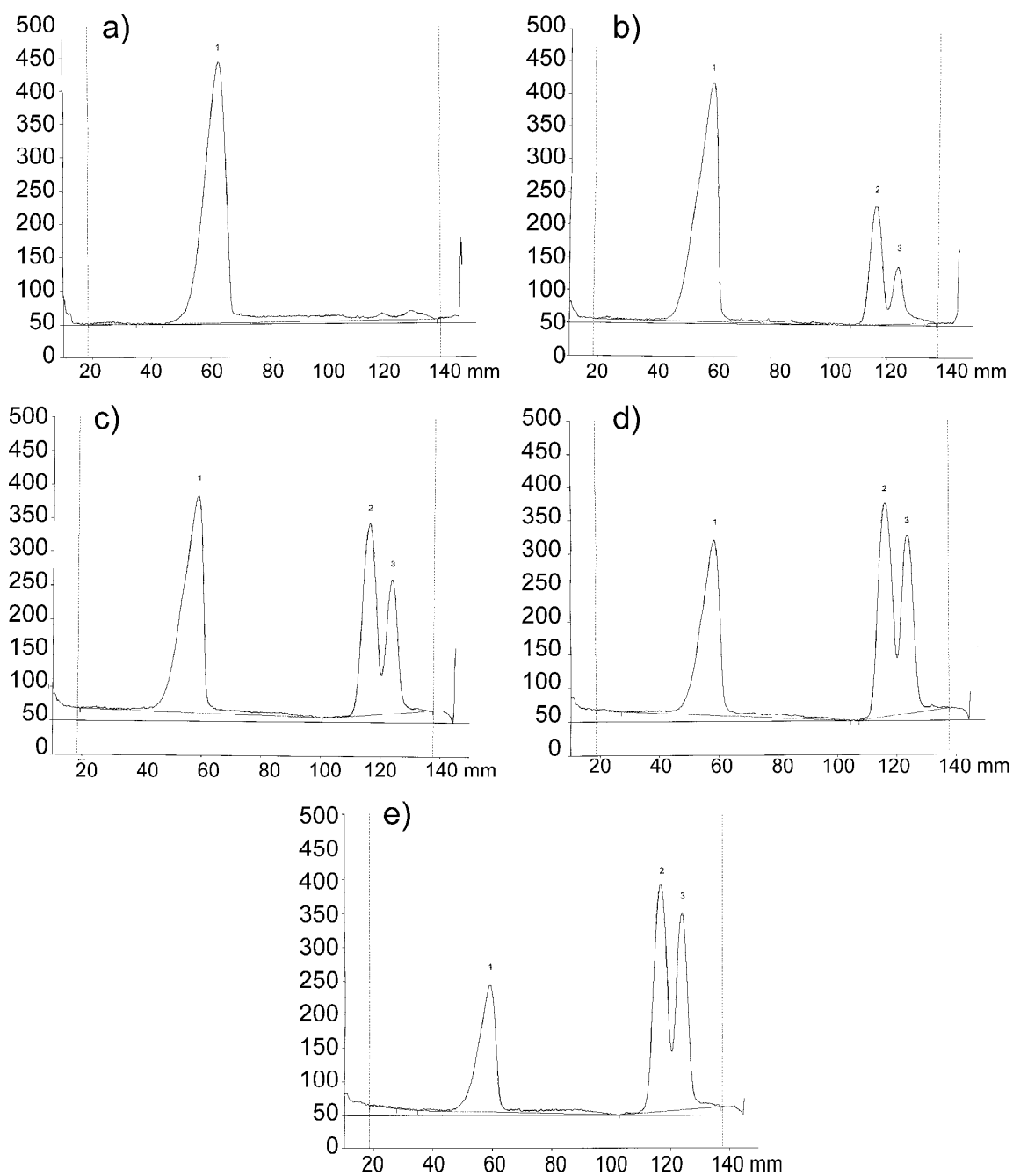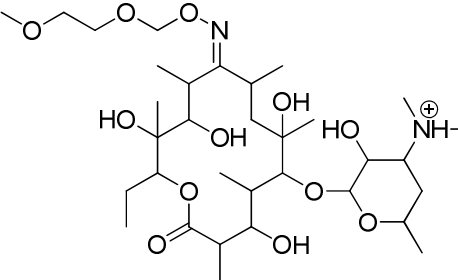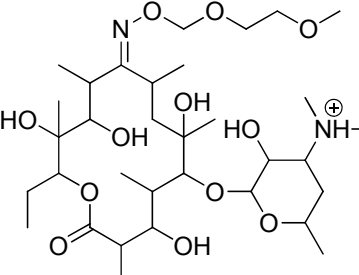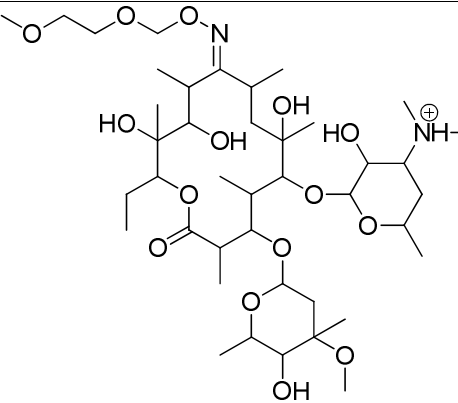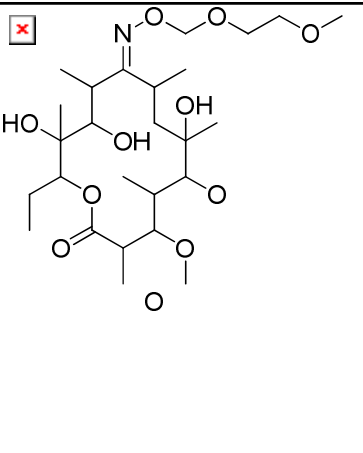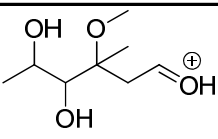
Figure 3. Densitograms registered for roxithromycin after hydrolysis at pH=1.70 at 60°C after 0 (a), 30 (b), 60 (c), 90 (d), and 120 (e) min. Peak 1 - roxithromycin, peak 2 and 3 - degradation products.

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Table 2. Proposed structures of the products of acidic hydrolysis of E-roxithromycin

Product Id	RT	[M+H]$^+$	Fragmentation ions	Proposed structure
RP-1	4.16	679.5	115.1, 158.1, 176.1, 398.2, 416.3, 446.3, 573.4, 603.4, 661.4	 m/z = 679.4
RP-2	4.39	679.5	115.1, 158.1, 176.1, 398.2, 416.3, 446.3, 573.4, 603.4, 661.4	 m/z = 679.4
RP-3	4.86	837.6	115.1, 158.1, 176.1, 398.2, 416.3, 446.3, 573.4, 603.4, 661.4, 679.4	 m/z = 837.5

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E-roxithromycin	5.06	837.6	115.1, 158.1, 176.1, 398.2, 416.3, 573.4, 603.4, 661.4, 679.4	
L-cladinose	6.16	177.1	161.1, 147.1, 149.1, 129.1, 119.1, 103.1, 101.1, 85.1, 75.1	

Structures of the presented stable products of the degradation were proposed based on Collisionally Activated Dissociation (CAD) experiments. The fragmentation pattern involved loss of L-cladinose substituent (-176.1) of E and Z-roxithromycin. In the next step decladinose derivatives lost H_2O (-18) and subsequently methoxyethene moiety (-58). These products underwent further decomposition on two routes: by deformylation (-30) and subsequentially loss of D-desosamine substituent, or loss of N,N,2-trimethyl-3,4-dihydro-2*H*-pyran-4-amine (-157.1), subsequent deformylation (-30) and loss of H_2O (-18) (see Supplementary Information Scheme S1).

D-Desosamine and its decomposition products were also observed. D-Desosamine underwent dehydration (-18) and subsequentially loss of N-methylenemethanamine fragment (-43) (see Supplementary Information Scheme S2). The main route of fragmentation of L-cladinose involved loss of carbon monoxide (-28) and subsequent deformylation (-30) and dehydration (-18). Other observed routes involved deformylation (-30) with subsequent dehydration (-18) and loss of acetaldehyde moiety (-44), demethylation (-14), or

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decarbonylation (-28) with subsequent loss of acetaldehyde moiety (-44) and decarbonylation

(-28) (see Supplementary Information Scheme S3).

Sensitized by TiO$_2$ Photodegradation of Roxithromycin

Although acidic hydrolysis of Roxy is quite efficient, it requires very low pH (<3) and

elevated temperatures so it may not be always applicable for Roxy hydrolytic degradation [7].

Therefore, we propose also an alternative method of Roxy degradation; TiO$_2$ sensitized

photocatalytic degradation. The photosensitizer in the form of a powder can be obtained in a

facile way by the hydrolysis of titanium (IV) isopropoxide and can be easily suspended in

water.

To find out which isomorphic form is the main constituent of the TiO$_2$ powder

obtained the Powder X-Ray Diffractometry (PXRD) measurement was performed. The

presence of Bragg peaks originating only from the anatase crystal structure in the PXRD

diffractograms confirm that it is the dominant crystal structure of the photocatalyst (see Figure

S1 in Supplementary Information). There are no peaks originating from rutile or brookite

indicating that these phases are absent, however, the presence of amorphous TiO$_2$ can not be

ruled out. Assuming spherical particle geometry, the average crystallite size calculated from

the line XRD line broadening is around 43 nm, thereby confirming its nanostructural

morphology.

The diameter of TiO$_2$ particles in water colloid found in the dynamic light scattering

measurements are around 900 nm (Figure S2 in Supplementary Information). It indicates that

TiO$_2$ crystallites form larger clusters. This effect might be attributed to the temperature

induced sintering of the grains during calcination step.

The bandgap energy estimated from the reflectance UV-Vis spectra equals to 3.13 eV

what further confirms the crystal structure of anatase. The wavelength of the photocatalyst

absorption band closely overlaps with the wavelength of the maximum intensity of the Rayonet lamps emission. On the other hand, the absorption band of Roxy, which ends at about 240 nm, is separated from the emission band of the lamps (see Figure S3 in Supplementary Information). This implies that the lamp radiation is strongly absorbed by the photocatalyst and its absorption by Roxy can be neglected.

Photocatalytic degradation studies of Roxy revealed that the drug studied undergoes photodegradation when irradiated with light at 350 nm (near UV) in the presence of TiO_2. Kinetics of the first order reaction follows the suggested theory of Langmuir-Hinshelwood for photocatalytic microheterogeneous systems (**Figure 4**) with the reaction rate constant of 0.00768 min^{-1}. Since Roxy does not absorb the light used for the irradiations the direct photolysis of Roxy did not occur.
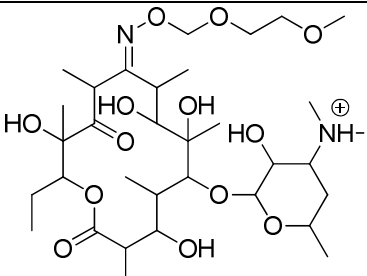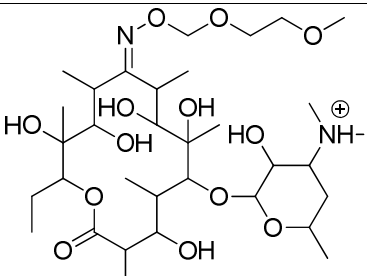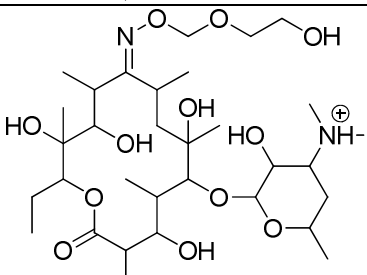

Figure 4. Photodegradation of roxithromycin in the absence and in the presence of TiO_2 photosensitizer.
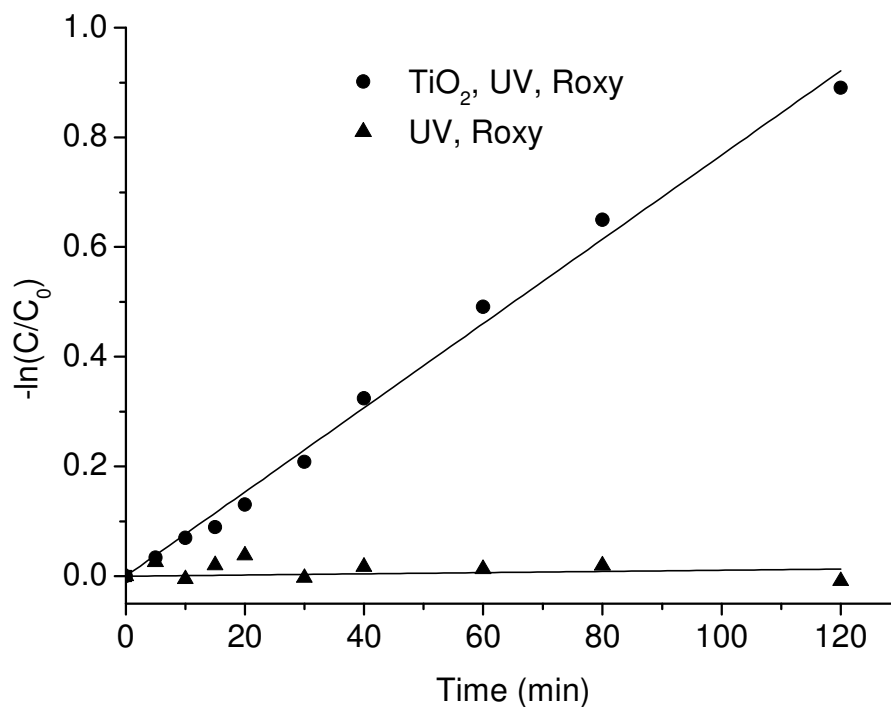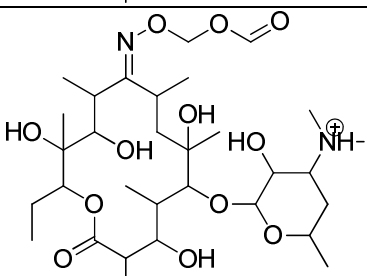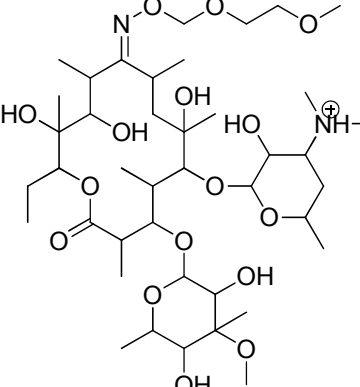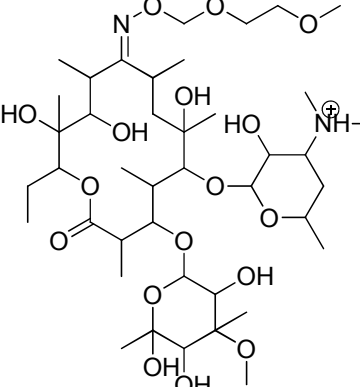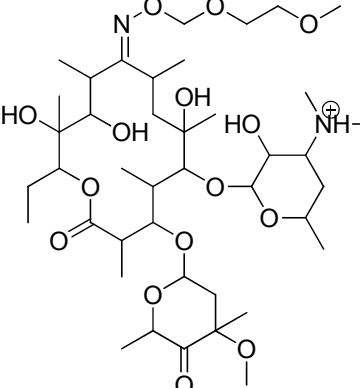
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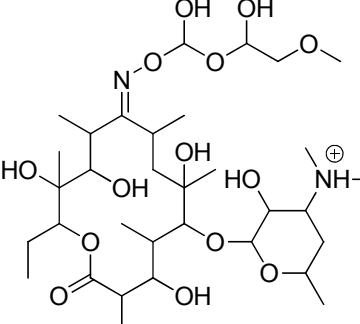14
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Adsorption process of Roxy on the surface of TiO_2 does not have a significant impact on the course of the experiment, as evidenced by minimal changes of the Roxy concentration for the experiment carried out in the dark, which also excluded acidic hydrolysis at this temperature.

The possible degradation products are shown in **Table 3.**

Table 3. Proposed products of photocatalytic degradation of E-roxithromycin in the presence of TiO_2

Product Id	RT	$[M+H]^+$	Fragmentation ions	Proposed structure
RP-UV-1	3.54	693.4	115.1, 158.1, 376.2, 500.3, 569.3, 587.4, 675.4	
RP-UV-2	3.70	695.4	115.1, 158.1, 571.3, 677.4	
RP-UV-3	3.88	665.4	115.1, 158.1, 490.3, 647.4	
RP-UV-4	4.38	649.4	115.1, 158.1, 398.2, 416.3, 446.3	

RP-UV-5	4.59	711.4	115.1, 158.1, 398.2, 470.3, 488.3, 675.4, 693.4	
RP-UV-6	4.74	853.5	115.1, 158.1, 398.2, 446.3, 573.4, 661.4, 679.4	
RP-UV-7	5.05	869.5	115.1, 158.1, 398.2, 679.4, 851.5	
RP-UV-8	5.48	835.5	115.1, 158.1, 176.1, 398.2, 446.3, 573.4, 603.4, 661.4, 679.4	

The photocatalytic degradation was found to affect mainly the L-cladinose substituent, causing its hydroxylation (RP-UV-6 and RP-UV-7) or oxidation of hydroxy substituent (RP-UV-8).

In the case of decladinose roxithromycin photocatalytic degradation seems to involve oxidation of 2-methoxyethoxymethyl moiety (products RP-UV-4 and RP-UV-5), demethylation of 2-methoxyethoxymethyl moiety (product RP-UV-3) or oxidation of the core of the antibiotic (RP-UV-1 and RP-UV-2).

Structures of the presented stable products of photocatalytic degradation were proposed based on Collisionally Activated Dissociation (CAD) experiments. The fragmentation patterns of products RP-UV-3, RP-UV-4 and RP-UV-6 – RP-UV-8 were similar to that observed for products of acidic degradation of Roxy. Additionally for RP-UV-7 fragmentation ion with m/z=851.5 was observed, which was probably the product of dehydration of 4-methoxy-2,4-dimethyltetrahydro-2H-pyran-2,3,5,6-tetraol moiety (2,5-dihydroxy-L-cladinose) to 5,6-dihydroxy-4-methoxy-2,4-dimethyldihydro-2H-pyran-3(4*H)*-one (2-hydroxy-4-oxo- L-cladinose). Fragmentation pattern for RP-UV-1 and RP-UV-2 is similar to previously described, but due to the presence of additional hydroxyl moiety in roxithromycin core it involves additional dehydration of fragmentation ions. The fragmentation path of RP-UV-1 is presented on **Scheme S4** (see Supplementary Information). In the case of RP-UV-5, possessing additional hydroxy substituents attached to the 2-methoxyethoxymethyl moiety, fragmentation involves dehydration of this moiety, besides of previously mentioned transitions. The fragmentation path for this product is presented on **Scheme S5** (see Supplementary Information). D-Desosamine and its decomposition products were also observed.

Conclusions

Detailed studies on the acidic hydrolytic degradation and TiO$_2$ sensitized photodegradation of roxithromycin have shown that both processes are quite efficient. Although the mechanisms of these processes are quite different, in both of them L-cladinose substituent is primarily involved. The structures of the degradation products and the mechanisms of the processes studied were proposed. Both types of the processes can be used for degradation of Roxy, however, the photocatalytic degradation seems to be more promising because it can undergo in mild conditions.

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