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Photodegradation kinetics of androgenic steroids boldenone and trenbolone in aqueous solutions

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ABSTRACT

The subject of this study was photodegradation of boldenone and trenbolone belonging to the class of compounds which demonstrate endocrine disrupting activity. Their decomposition was carried out on the pathway of 254 nm photolysis alone and in the presence of hydrogen peroxide. The influence of pH, oxygen content in the reaction mixture, initial substrate concentration, photon fluence rate and initial concentration of H_2O_2 on the reaction rate was investigated. The effect of radical scavenger on the reaction course was also tested. The obtained results were used to determine kinetic parameters. Quantum yields of boldenone and trenbolone decay were equal to 0.61 ± 0.02 and 0.0029 ± 0.0002, respectively. The estimated rate constant of the reaction of hydroxyl radicals with trenbolone was $(4.3 \pm 0.8) \times 10^9\ M^{-1}\ s^{-1}$.

1. Introduction

Currently, numerous scientific researches indicate that some substances in the environment may interfere with a normal function of the endocrine system of humans and wildlife [1,2]. Minute amounts of these chemicals are able to disrupt the endocrine system and cause cancer to male and female reproductive systems and produce other adverse effects. Therefore, these substances are called endocrine disruptors (EDs).

A particular group of EDs consists of pharmaceuticals based on steroid moiety both of natural and synthetic origin. They are mainly used as contraceptives, drugs applied in menopause, breast cancer and for other medical purposes. A further important group of pharmaceutical hormones are anabolic steroids used in agriculture in animal farming. In EU countries application of growth-promoting hormones is banned in meat production [3] but in other countries they are regularly applied (e.g. USA or Canada) [4]. There is a growing concern that these synthetic hormones are making their way into surface waters and even ground water via human and animal wastes, mainly by their incomplete removal during passage through wastewater treatment plants [4,5]. Natural and synthetic hormones such as 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) are frequently detected in the nanogram per liter ranges [6,7].

Compared with the natural hormones, synthetic hormonal steroids show relatively greater stability in aqueous media and greater resistance to microbial degradation [4]. These properties pose a potential for accumulation and persistence in the environment and endanger consumers with a permanent exposure. It can be presumed that other structurally related xenobiotic hormones used in veterinary treatment show a similar behavior.

Boldenone and trenbolone belong to a group of synthetic steroids with great anabolic potency. Their chemical structures are shown in Fig. 1.

Boldenone (BD) is a steroid which differs from testosterone only by one double bond at the 1-position. It is used mainly as undecylenate ester by bodybuilders and is illicitly administered to racing horses. But first of all it is applied as a growth promoter on meat production farms improving the growth and feed conversion of cattle and therefore might be abused to achieve more efficient meat production. Like other androgenic steroids, BD is classified as a probable human carcinogen [8].

Trenbolone (TB) is characterized by a very high anabolic effect nearly 10 times stronger than that of testosterone propionate [4] and is licensed as a growth promoter in the US and Canada. It is suspected of teratogenic activity as it can induce developmental abnormalities in rat's fetus [9]. The demasculinizing effect in Japanese quail was observed [10] and reproductive alterations in fish living downstream from animal feedlot operations were reported [9]. The problem with TB is more evident taking into account that in the US only several tons is applied every year [4] and that TB can remain in manure piles for more than 270 days [5].

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Nomenclature

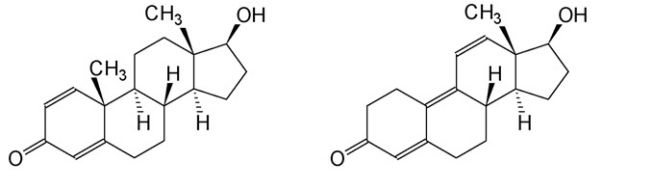
B	optical path length
BD	boldenone
C_i	molar concentration
C_i^0	initial molar concentration
F	fraction of absorbed photon flux
H	hydrogen peroxide
HO_2	hydroperoxide ion
E_0	volumic photon fluence rate
K	dissociation constant
k	rate constant
k_z	I-order apparent rate constant
OH	hydroxyl radical
R	reaction rate
t	time
TB	trenbolone
ε	molar absorption coefficient
λ	wavelength
φ	quantum yield
0	initial conditions

The studies on degradation of hormone steroids in aqueous solutions were performed mainly for estrogens such as estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2) and estriol (E3). There were various methods applied for their degradation. Ozonation and ozone-based advanced oxidation processes [6] resulted in efficient removal of the estrogen steroid from the aqueous environment. Worth noting is a very high rate constant of the direct reaction of ozone with dissociated forms of steroids attaining diffusion controlled limit which is 10^4 to 10^5 times greater than with neutral forms of steroids [11]. The application of OH radicals using the H_2O_2/UV system to eliminate the estrogen steroids from aqueous solutions is characterized by rate constants of the order 10^{10} M^{-1} s^{-1} [12,13]. However, photolysis as well as the use of high energy radiation of a low pressure lamp (254 nm) as solar or solar-simulated light is reported to be inefficient [14]. The determined quantum yields are of the order 10^{-2} reaching 0.1 for E2 at radiation from a medium pressure lamp [13]. The direct photolysis using 254 nm radiation can be enhanced in natural waters due to the presence of various photo-oxidants [15].

The present paper describes aqueous BD and TR degradation studies during 254 nm irradiation alone and in the presence of hydrogen peroxide. The obtained results were used to estimate the decay quantum yield of both studied compounds and rate constant of the reaction of hydroxyl radicals with TB.

2. Materials and methods

Boldenone (BD) (98.8%) was purchased from Sigma–Aldrich, trenbolone (TB) (98%) and *tert*-butyl alcohol (≥99.7%) were from Fluka. Hydrogen peroxide (30% solution), methanol (p.a.) and



Boldenone, MW 286.41
1,4-androstadiene-3-one-17β-ol

Trenbolone, MW 270.37
17β-hydroxyestra-4,9,11-trien-3-one

Fig. 1. Chemical structures of studied steroids.

reagents used to prepare buffers (Na_2HPO_4, KH_2PO_4, NaOH, p.a.) were bought from POCH (Poland).

Experiments were carried out in quartz test tubes of the capacity of 10 cm^3 (optical path length 0.85 cm), placed in a merry-go-round device which was located between two exposure panels with UVC lamps emitting mainly wavelength at 254 nm. The initial concentrations of the substrates ranged from 4.8×10^{-6} to 1.8×10^{-5} M and from 5×10^{-6} to 1×10^{-4} M for BD and TB, respectively. The details of equipment, the run of the process and the reaction mixture preparation were analogous to those outlined elsewhere [16]. Photon fluence rate entering the reaction space was calculated by uranyl oxalate actinometer [17] and ranged from 4.3×10^{-6} to 10.6×10^{-6} einstein dm^{-3} s^{-1} for 2 and 6 lamps, respectively. These fluence values corresponded to irradiance from 11.8 to 29.1 W m^{-2}.

Spectrophotometric measurements were done on a Unicam apparatus.

The reaction progress was traced by chromatographic analysis of BD and TB decay. The HPLC (Waters) apparatus equipped with a UV diode array detector and Symmetry C18 reverse-phase column, operated in isocratic mode was used. The mobile phase consisted of 30% acetonitrile and 70% of 0.01% (v/v) water solution of phosphoric (V) acid and the flow rate 1 cm^3 min^{-1} was applied.

Data analysis was performed using Origin software (Microcal Software Inc., USA). The initial reaction rates were calculated by differentiating exponential curve that fitted experimental points (concentration, time) at the correlation factor higher than 0.97.

3. Results and discussion

The spectrophotometric measurements of BD and TB as the basis of photochemical studies were performed and molar absorption coefficients were determined. The absorption spectra of BD and TB in water solutions and emission spectrum of the UVC lamp are shown in Fig. 2.

The maximum of the BD absorption appears at 252 nm which fits very well with the emission of the UVC lamp. TB absorption extends in the whole near-UV range up to nearly 500 nm in the visible range, with two maxima: clear one at 351 nm and broad band centered at 237 nm. The determined molar absorption coefficient ε at 254 nm for BD at pH 5, 7 and 10 is equal to 13,800, 14,570 and 15,240 M^{-1} cm^{-1}, respectively, and for TB it is 5460, 6300 and 5690 M^{-1} cm^{-1}, respectively.

The absorption of both compounds slightly depends on pH of the solution, probably due to the interaction of BD and TB chromophores with the solution components. BD and TB can dissociate,

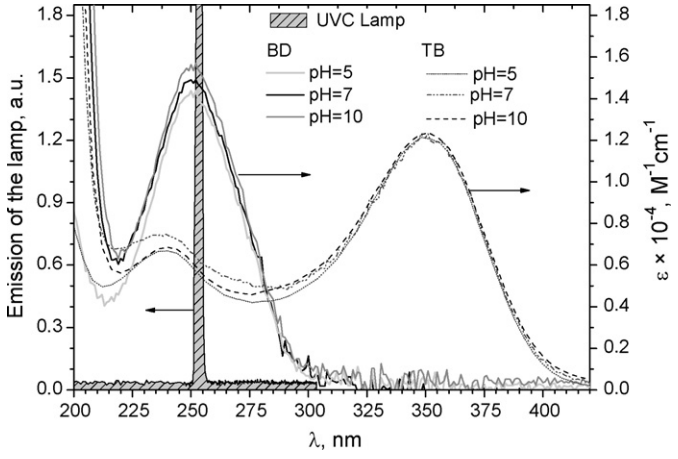


Fig. 2. The absorption spectra of BD and TB in buffered reaction solutions and the emission spectrum of the UVC lamp.

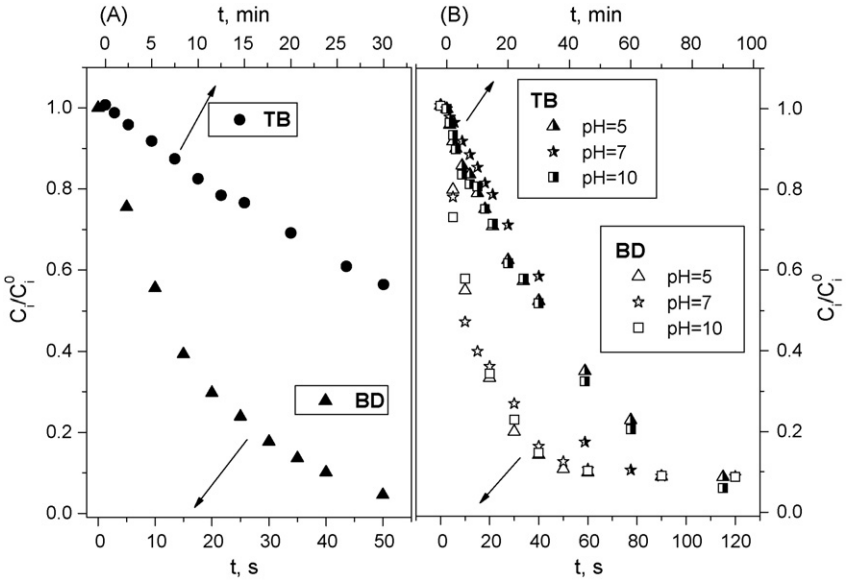


Fig. 3. (A) Disappearance of BD and TB during photolysis (pH 7; $C^0_{BD} = 1 \times 10^{-5}$ M, $E_0 = 4.3 \times 10^{-6}$ einstein dm^{-3} s^{-1}; $C^0_{TB} = 1 \times 10^{-5}$ M, $E_0 = 10.6 \times 10^{-6}$ einstein dm^{-3} s^{-1}). (B) The influence of solution pH on the decay of BD and TB during photolysis ($C^0_{BD} = 1.8 \times 10^{-5}$ M, $E_0 = 4.3 \times 10^{-6}$ einstein/dm^{-3} s^{-1}; $C^0_{TB} = 5 \times 10^{-5}$ M, $E_0 = 10.6 \times 10^{-6}$ einstein/dm^{-3} s^{-1}).

but are very weak acids as can be inferred from pK_a values for other steroids [7].

3.1. Direct photolysis

The degradation of BD during photolysis by 254 nm radiation was much faster than that of TB (Fig. 3A). Starting from the BD concentration of 1×10^{-5} M, a 50% reduction was achieved in 11 s using fluence rate equal to 4.3×10^{-6} einstein dm^{-3} s^{-1}, whereas the TB of the same initial concentration was halved in 30 min using fluence rate 2.5 times higher (10.6×10^{-6} einstein dm^{-3} s^{-1}). Such a significant difference in the reaction rates was to some extent a result of various values of molar absorption coefficients of BD and TB at 254 nm.

The 50% degradation of steroids was achieved after exposure of the reaction solutions to 130 J m^{-2} and about 52,000 J m^{-2} energy for BD and TB, respectively. Comparing these values with UV disinfection dose usually applied for tap water treatment equal to 400 J m^{-2} [15], one can notice that only boldenone could be removed in the disinfection process. Especially, employing higher irradiance of a low-pressure UV lamp, a complete BD removal can be achieved in several seconds. In the case of TB, a probably better removal degree should be attained using a medium-pressure UV lamp.

The pH of reaction medium often influences the photoreaction rate due to various electron distributions in the molecules. The photolysis of steroids was studied at three pH values: 5, 7 and 10. The obtained results show a very weak or no influence of pH on the decay rate of studied compounds, which is illustrated in Fig. 3B.

The photolysis of steroids is independent of oxygen presence in the reaction mixture. The experiments carried out with a solution saturated with air and nitrogen practically did not show any differences (Fig. 4A). Hence the initial decay of BD and TB can proceed without the participation of oxygen. In the degradation of steroids the hydroxyl radicals did not take part either, which was indicated by the process run in the presence of *tert*-butyl alcohol—the known hydroxyl radical scavenger (Fig. 4A).

The plot in Fig. 4B shows the course of photolysis for various initial concentrations of steroids. An increase in the concentration resulted in a higher rate of the reaction. This relation is almost directly proportional in the studied concentration range.

The absorption of UV radiation by the examined compounds causes their decay according to the reaction:

$$\frac{\text{BD}}{\text{TB}} + h\nu \rightarrow \text{products} \quad (1)$$

For the initial reaction time, when the molecules are solely absorbing species, the reaction rate can be expressed using the following expression:

$$r = -\frac{dC_i}{dt} = \phi_i E_0 \left[1 - \exp(-2.303 b \varepsilon_i C_i)\right] \quad (2)$$

This relationship was used to calculate decay quantum yields ϕ_i of BD and TB. In the studied range of steroids concentrations at neutral pH of the reaction solution, the value of φ was equal to 0.61 ± 0.02 and 0.0029 ± 0.0002 for BD and TB, respectively. In the alkaline solution quantum yields were roughly estimated to be 0.61 and 0.0038 for BD and TB, respectively. The curves in Fig. 4B show good correlation with experimental data.

3.2. Decomposition of TB by H_2O_2/UV process

The experiments in the H_2O_2/UV system were performed only for TB solution because in the case of BD the reaction was very fast and measurements of the reaction progress were hindered even at the lowest fluence rate. The highest rate of TB decay was achieved at hydrogen peroxide concentration equal to 1×10^{-2} M. Above this value the reaction rate dropped rapidly. This decline was a consequence of scavenging hydroxyl radicals by the reaction with H_2O_2. The verification of the effect of fluence rate on TB decay confirmed a typical rectilinear relationship.

The addition of hydrogen peroxide to the reaction solution of TB caused a significant acceleration of its photodegradation (Fig. 5A). The half-life of TB during the direct photolysis at fluence rate 4.3×10^{-6} einstein dm^{-3} s^{-1} was about 120 min, whereas addition of H_2O_2 at optimal concentration ($C^0_H = 1 \times 10^{-2}$ M) reduced it to about 90 s.

Studies of TB degradation in the UV/H_2O_2 system were also performed in the presence of a hydroxyl radical scavenger. In these

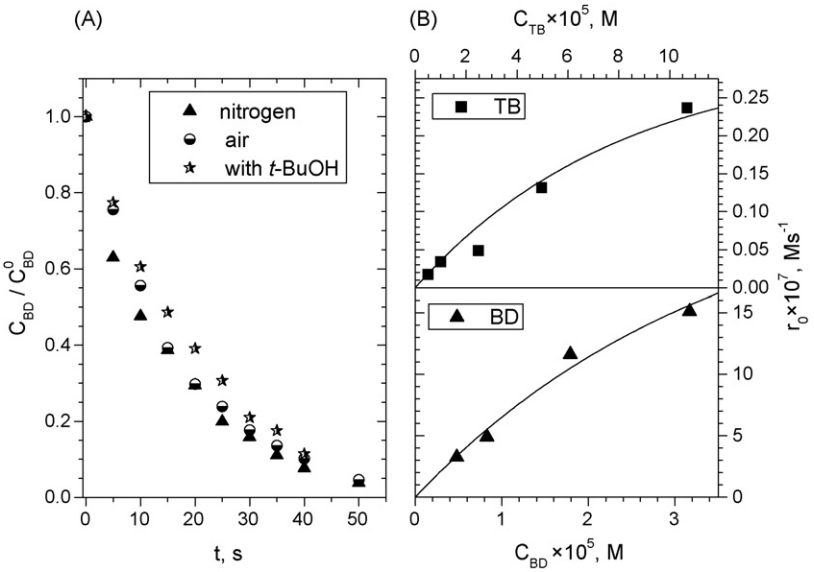


Fig. 4. (A) The effect of *tert*-butanol presence and oxygen content in the reaction solution on the decay of BD ($C^0_{BD} = 1.8\times10^{-5}$ M, $C^0_{t\text{-BuOH}} = 1\times10^{-1}$ M, pH 7, $E_0 = 4.3\times10^{-6}$ einstein dm^{-3} s^{-1}). (B) The influence of the initial concentration of BD ($E_0 = 4.3\times10^{-6}$ einstein dm^{-3} s^{-1}) and TB ($E_0 = 10.6\times10^{-6}$ einstein dm^{-3} s^{-1}) on the initial reaction rate during photolysis at pH 7. Points represent experimental data and lines represent the plot of expression (2).

tests the *tert*-butyl alcohol was used as a radical scavenger. The results show inhibition of the decomposition rate of TB (Fig. 5A), which confirmed the dominating role of hydroxyl radicals reaction in this process.

Fig. 5B shows the dependence of initial reaction rate on starting TB concentration. The rising TB concentration yielded an increase of the observed decay rate, after that the reaction slowed down. This observation can be explained by a competition between hydrogen peroxide and TB of UV radiation. At a low TB concentration photons were absorbed mainly by H_2O_2 causing production of a hydroxyl radical and at an increasing TB concentration leading to a higher reaction rate. At the higher concentration, TB became the main absorber of photons causing a decrease of hydroxyl radical concentration and inhibition of the reaction rate. At the TB concentration 1×10^{-5} M it absorbs above 25% of radiation, at the concentration equal to 1×10^{-4} M it absorbs above 77% of radiation leaving only 23% for hydrogen peroxide. The molar absorption coefficient of H_2O_2 at 254 nm is about 340 times smaller than that for TB (18.6 M cm^{-1} [18] and 6300 M cm^{-1}, respectively).

The TB degradation was also performed in solutions of various pH. The obtained results indicate an inhibiting action of a rising pH on the reaction rate (Fig. 6A). This is caused by an increasing dissociation degree of hydrogen peroxide (pK_a = 11.6 [19]). The hydroperoxide anion is an efficient scavenger of hydroxyl radicals, better than hydrogen peroxide (see Table 1), which leads to diminishing the concentration of hydroxyl radicals available for the reaction with TB.

The photodegradation of TB by hydrogen peroxide combined with UV radiation can be treated as a process consisting of direct photolysis and reaction with hydroxyl radicals generated in H_2O_2

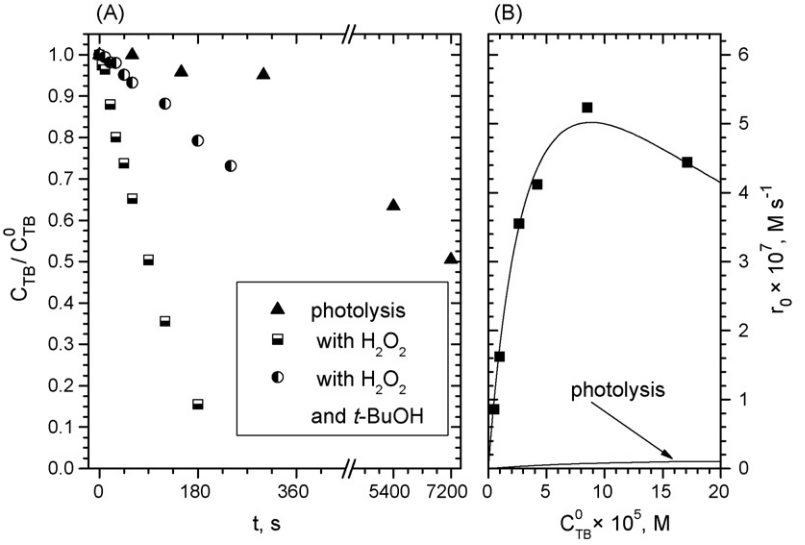


Fig. 5. (A) The comparison of reaction run during photolysis and in the H_2O_2/UV system in the presence and absence of *t*-butanol ($C^0_{TB} = 5.2\times10^{-5}$ M, $C^0_H = 1\times10^{-2}$ M, $C^0_{t\text{-BuOH}} = 1\times10^{-2}$ M, pH 7, $E_0 = 4.3\times10^{-6}$ einstein dm^{-3} s^{-1}). (B) The dependence of initial reaction rate of TB decay on its initial concentration. The curve represents calculated total reaction rate of photolysis and reaction with •OH radicals, using Eqs. (6), (7) and (11). Bottom curve represents the contribution of photolysis alone (Eq. (7)) in the total rate of TB decay ($C^0_H = 1\times10^{-2}$ M, pH 7, $E_0 = 4.3\times10^{-6}$ einstein dm^{-3} s^{-1}).

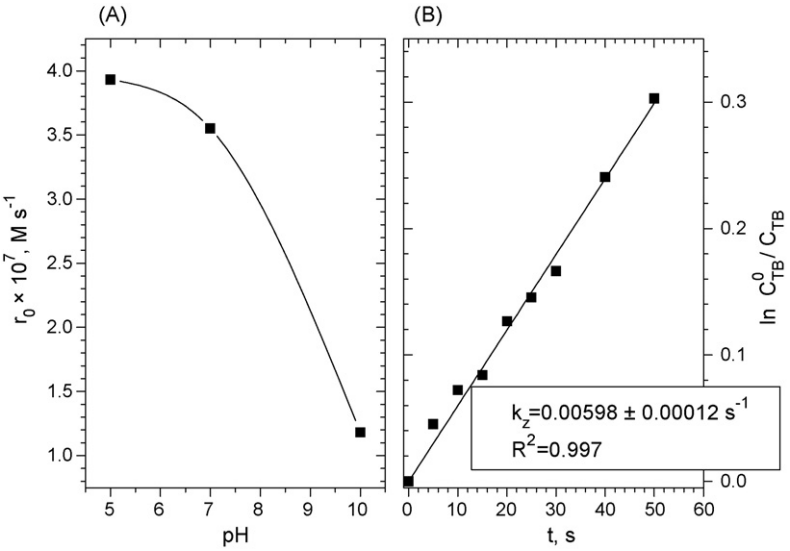


Fig. 6. (A) The influence of the pH of reaction mixture on the initial rate of TB decay in the H_2O_2/UV system ($C^0_{TB} = 2.5 \times 10^{-5}$ M, $C^0_H = 1 \times 10^{-2}$ M, $E_0 = 4.3 \times 10^{-6}$ einstein dm^{-3} s^{-1}). (B) Determination of pseudo-first order rate constant for TB decay in the H_2O_2/UV process (pH 7, $C^0_{TB} = 2.36 \times 10^{-5}$ M, $C^0_H = 1 \times 10^{-1}$ M, $E_0 = 4.3 \times 10^{-6}$ einstein dm^{-3} s^{-1}).

photolysis. In this system the following reactions can occur:

$$H_2O_2 + h\nu \rightarrow 2^{\bullet}OH \quad 2\phi_H E_0 F_H \left[1 - \exp\left(-2.303b \sum \varepsilon_i C_i\right)\right] \quad (3)$$

$$^{\bullet}OH + H_2O_2 \rightarrow H_2O + HO_2^{\bullet} \quad k_4 C_H C_{OH} \quad (4)$$

$$^{\bullet}OH + HO_2^- \rightarrow H_2O + O_2^{\bullet -} \quad k_5 C_{HO_2} C_{OH} \quad (5)$$

$$TB + {}^{\bullet}OH \rightarrow \text{products} \quad k_{OH} C_{OH} C_{TB} \quad (6)$$

$$TB + h\nu \rightarrow \text{products} \quad \phi_{TB} E_0 F_{TB} \left[1 - \exp\left(-2.303b \sum \varepsilon_i C_i\right)\right] \quad (7)$$

When the process is carried out with high concentration of hydrogen peroxide such that it absorbs all incident radiation, then TB photolysis can be neglected. Kinetic calculations were done for the data obtained from the experiment when hydrogen peroxide concentration was equal to 0.1 M and pH equal to 7. In this condition the fraction of UV radiation absorbed by H_2O_2, according to expression (8) was 0.93.

$$F_H = \frac{\varepsilon_H C_H}{\Sigma \varepsilon_i C_i} \quad (8)$$

The rate of TB photolysis alone in these conditions, calculated according to Eq. (7) was equal to 1.1×10^{-9} M s^{-1} and the rate of TB decay, calculated from experimental data was equal to about 1.3×10^{-7} M s^{-1}, which confirms that it was justified to neglect the direct TB photolysis.

Simultaneously, the hydrogen peroxide concentration at initial stages of the reaction can be treated as constant. In this case, the rate of TB decay can be expressed as the equation at the right-hand side of (6). Additionally, in these circumstances the assumption of steady state for hydroxyl radical concentration allowed us to express the rate law as the pseudo-first order kinetics:

$$-\frac{dC_{TB}}{dt} = k_z C_{TB} \quad (9)$$

where

$$k_z = k_{OH} C_{OH} \quad (10)$$

is the pseudo-first order rate constant. The plot of integrated form of Eq. (9) should follow a straight line in the coordinate system $\ln(C_o/C) - t$. Fig. 6B shows the verification of this relationship and calculated slope of the line which corresponds to k_z. At the beginning of the reaction, the produced hydroxyl radicals attack TB, but as the reaction proceeds, the substrate concentration is depleted. This can lead to the deviation from steady-state $^{\bullet}OH$ concentration. However, hydroxyl radicals react also with formed intermediates and probably at the same or very close rate constants as TB. Thus, the steady-state assumption remains valid also at higher conversion [20].

It follows from the steady state assumption that the formation and disappearance rates of hydroxyl radicals are equal to each other. If we combine these rate equations (3)–(6) we will obtain the expression of hydroxyl radicals concentration:

$$C_{OH} = \frac{2\varphi_H E_0 F_H \left[1 - \exp\left(-2.303b \sum \varepsilon_i C_i\right)\right]}{k_4 C_H + k_5 C_{HO_2} + k_{OH} C_{TB}} \quad (11)$$

The presence of phosphates used as buffers in the reaction solution does not practically affect the hydroxyl radical concentration. The rate constants of $^{\bullet}OH$ with HPO_4^{2-} (1.5×10^5 M^{-1} s^{-1} [21]) and with $H_2PO_4^-$ (2×10^4 M^{-1} s^{-1} [21]) at applied concentrations 0.022 and 0.028 M, respectively, do not cause any change in the value of the denominator in Eq. (11).

Combining Eqs. (10) and (11) and after rearrangement we get:

$$k_{OH} = \frac{k_z(k_4 C_H + k_5 C_{HO2})}{2\phi_H E_0 F_H \left[1 - \exp\left(-2.303b \sum \varepsilon_i C_i\right)\right] - k_z C_{TB}} \quad (12)$$

Table 1
The values of constants used in calculations of rate constants of TB degradation and obtained results.

Constant	ε_{TB} at 254 nm M^{-1} cm^{-1}	ε_H at 254 nm M^{-1} cm^{-1}	pK_a for H_2O_2	φ_H	k_4 (M^{-1} s^{-1})	k_5 (M^{-1} s^{-1})	k_{OH} (M^{-1} s^{-1})
Value	6300	18.6	11.6	0.5	2.7×10^7	7.5×10^9	$4.3 \pm 0.8 \times 10^9$
Source	This work	[18]	[19]	[22]	[23]	[23]	This work

Relationship (12) allowed us to calculate the value of second-order rate constant of hydroxyl radical reaction with TB. The values of constants necessary for calculations and the obtained results are given in Table 1.

The dependence of reaction rate upon TB concentration calculated from Eqs. (6), (7) and (11), using determined rate constant and quantum yield, shows quite good correlation with the reaction rates evaluated from experimental points, which is presented in Fig. 5B.

4. Conclusions

The photolysis of BD and TB initiated by 254 nm radiation leads to destruction of both steroids. In the case of BD, photolysis occurs very fast and is independent of pH of the reaction solution. The complete removal of BD is attained in 1 min at irradiance 11.8 W m^{-2}. This high rate of BD decay indicates a possibility of its removal during UV disinfection of drinking water. The determined quantum yields of BD and TB decay are equal to 0.61 and 0.0029, respectively. In alkaline solution the photolysis of TB is slower. For BD and TB the content of oxygen in aqueous solution does not influence the photolysis rate and the process proceeds without the participation of hydroxyl radicals.

The addition of hydrogen peroxide during photolysis significantly accelerates the rate of steroids decay. In the case of BD the rate became so high that it was impossible to measure the reaction progress in the used experimental system. The highest TB degradation rate in the H_2O_2/UV system was achieved in acidic solutions and the rising pH resulted in the rate reduction. For given operating parameters (H_2O_2 concentration, fluence rate) the maximum process rate for optimal TB concentration is caused by a high value of the TB molar absorption coefficient. So, it is advisable to control TB concentration during its degradation in the H_2O_2/UV system.

The results of performed investigations made it possible to build a kinetic model and determine the rate constant of the reaction of hydroxyl radicals with TB equal to $(4.3 \pm 0.8) \times 10^9$ M^{-1} s^{-1}.

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