Wavelength-dependent photochemistry of acetaminophen in aqueous solutions

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The influence of irradiation wavelength and intensity on photochemistry of acetaminophen (APAP) in aqueous solution was investigated by combination of steady-state and laser flash photolysis as well as HPLC and LC–MS. Steady-state irradiation at 254 nm leads to APAP disappearance with the quantum yield 0.0014 and to formation of 1-(2-amino-5-hydroxyphenyl)ethanone (P1) as a main primary photo-Fries product. In opposite the laser excitation at 266 nm leads predominantly to two-photon ionization of APAP with the quantum yield 0.013 (1 \textminus 70 mJ/cm\textsuperscript{2}) and to the formation of one main product of phenoxyl radical reactions – N-(3,4-dihydroxyphenyl)acetamide (P5). Steady-state excitation at 282 nm leads to both P1 and P5 products formation indicating competition of photo-Fries and photoionization processes. The wavelength-dependent mechanism of APAP photolysis is proposed and discussed.

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1. Introduction

In the recent decades, pharmaceuticals and personal care products (PPCPs) in the environment is emerging as a new environmental concern for the scientists and public stakeholders. There are still some residual parts of PPCPs and their metabolites get into the surface and groundwater during and after the sewage treatment [1–6]. In particular, acetaminophen (paracetamol, abbreviated as APAP), a typical kind of PPCPs, which is widely used as an analgesic/antipyretic drug. It was found 58–68\% of APAP was excreted from the body during therapeutic use and a median concentration of 0.11 \mu\text{g}l\textsuperscript{-1} was detected in streams [1,7].

The oxidation and degradation of APAP was widely studied by \gamma\textsuperscript{-}radiolysis [8], UV irradiation in presence of TiO\textsubscript{2} and H\textsubscript{2}O\textsubscript{2} [9–12] or (bio)chemical oxidation [13–15]. In all cases the main primary species was APAP phenoxyl radical (RO\textsuperscript{•}) which decays with the formation of coupling, polymeric and hydroxylation products [10,11,13,14]. On the other side, direct UV photolysis of APAP is less studied. In recent papers it was shown that primary product of 254 nm photolysis of APAP is a product of photo-Fries reaction – 1-(2-amino-5-hydroxyphenyl)ethanone (P1) [16,17]. This reaction occurs from the singlet excited state of the molecule and involves the migration of the acetyl group onto the aromatic ring in the ortho-position to the amine moiety (reaction 1 [17]). The same mechanism was proposed for other para-substituted acetanilides [18]. This finding was in contradiction with the results of our work in which photoionization with formation of RO\textsuperscript{•}- hydrated electron pair was postulated as a main photochemical process based on data of nanosecond laser flash photolysis at 266 nm [19]. It is indicating that either the light intensity or the irradiation wavelength takes responsibility for the different degradation channels. Actually, although the UV photolysis of various PPCPs have been widely studied, most of the works were investigated under the irradiation at 254 nm and were focused on the effect of aquatic environments (pH, dissolved organic matter, exogenous ions, etc.) on the photo-transformation of the target pollutants. The effects of irradiation
conditions on the primary and secondary photochemistry of the investigated compounds were seldom explored.

So in this work mechanism of direct APAP photolysis was reinvestigated in detail by combination of steady-state (stationary photolysis, HPLC, LC–MS) and time-resolved (laser flash photolysis) methods. The main attention was paid to determination of APAP photolysis quantum yields and product’s nature, stability and distribution. It was found that all aforesaid parameters depend on both excitation wavelength and intensity of irradiation.

2. Experimental

2.1. Chemicals

Acetaminophen (98%) was purchased from Alfa Aesar and was used without further purification. 1-(2-Amino-5-hydroxyphenyl)ethanone (P1) was synthesized as described in a previous report [20] and have a purity about 97% by HPLC and 1H NMR. Sodium persulfate (chemically pure), LiClO₄ (Aldrich), HClO₄ (Aldrich) and acetonitrile (HPLC grade) were used without further purification. Absorption spectra and structure of APAP and P1 are shown in Fig. 1. APAP concentration was in range (6–50) × 10⁻⁵ M. The reaction solutions were prepared by doubly distilled water. Unless otherwise specified, all photochemical experiments were performed in a 1 cm quartz cell in air-equilibrated solutions at initial pH 6.5, temperature 298 K and atmospheric pressure.

2.2. Laser flash photolysis

The laser flash photolysis setup based on an LS-2137U Nd:YAG laser (Lotis TII, Belarus) with excitation wavelength of 266 nm, pulse duration of 5–6 ns, illumination spot area of 0.03 cm², and energy per pulse up to 10 mJ was used in the time-resolved experiments; the device was similar to that described in previous work [21]. Time resolution of the setup was ca. 50 ns. For steady-state irradiation at 254, 282 and 266 nm Hg low pressure lamp with chloride and water cut-off filters, XeBr excimer lamp and 4th harmonic of Nd:YAG laser were used, accordingly. Lamps and laser intensity was determined by using ferrioxalate actinometer in the same conditions as were used for HPLC measurements. The quantum yield of APAP photolysis was calculated from the initial linear decrease of APAP concentration with irradiation time, experiments were done in duplicate, and precision was ca. 20%.

2.3. HPLC analysis

The concentration of APAP in photolyzed solutions was determined by HPLC with UV-detection at 220 nm. HPLC experiments were performed using liquid microcolumn chromatograph Milichrom A-02 with ProntoSIL 120-5-C18 AQ #1810 column, 2.0 mm × 75 mm, 5 μm. The eluent was a mixture of acetonitrile with aqueous buffer solution (0.2 M LiClO₄ and 0.005 M HClO₄), gradient 5–100% acetonitrile. Flow rate was 100 μl/min, sample volume was 15 μl, and column thermostat temperature was 40 °C.

2.4. LC–MS and LC–MS/MS analysis

LC–MS/(MS) experiments were performed on ESI-q-TOF high-resolution hybrid mass-spectrometer Maxis 4G (Bruker Daltonics, Germany) with the HPLC-separation system UltiMate 3000RS (Dionex, Germany) equipped with ternary pump and diode array UV detection (DAD) in 220–400 nm range. Separation was performed on an analytical column Zorbax XDB-C18, 4.5 mm × 150 mm, 5 μm in the gradient of acetonitrile/0.1% formic acid: 10% (0–2 min), 10–80% (2–20 min), 80–95% (20–21 min), 95% (21–25 min), 95–10% (25–26 min), 10% (26–40 min). Flow rate was 200 μl/min, sample volume was 5 μl, and column thermostat temperature was 40 °C. The instrumental setup allows recording both DAD and MS data simultaneously. Experimental parameters: registration of ions was in the positive mode, range was 50–700 m/z, HV capillary was 4200 V, end plate offset was ~500 V, ESI nebulizer pressure was 1.0 bar, dry gas flow (N₂) was 8 l/min, temperature was 200 °C. The instrument was calibrated before each LC–MS/(MS) run with the infusion of the mixture containing sodium formate clusters via switching valve and syringe with the constant flow rate. The acquisition of fragmentation mass spectra (LC–MS/(MS)) was performed in automatic mode, picking two most abundant ions to be the parent ions for further isolation and fragmentation. After acquiring three good fragment spectra for an ion, the isolated
parent ion was released and the next most abundant ion was picked for the next MS/MS spectra acquisition in LC run.

3. Results and discussion

3.1. Laser flash photolysis of APAP at 266 nm

In our previous paper [19] it was shown that flash excitation of APAP at 266 nm leads to its photoionization with formation of hydrated electron ($\lambda_{\text{max}} = 720$ nm) – phenoxyl radical (RO*). This reaction is typical behavior for phenols in aqueous solution [22–24]. It was found that RO* decays in self-reaction ($2k_{\text{RO}^*} = 3.3 \times 10^3 \text{ cm} \cdot \text{s}^{-1}$) and with the superoxide radical ($k = 9 \times 10^5 \text{ M}^{-1} \text{s}^{-1}$), which is formed by quenching of the hydrated electron by dissolved oxygen [19].

In order to obtain additional evidence of RO* formation at 266 nm photolysis this species was generated by approach similar to be described in Bispy’s work [8]. The oxidation of APAP by photochemically generated one-electron oxidizer $SO_4^{+}$ was done [25].

$$S_2O_8^{2-} (266 \text{ nm}) \rightarrow SO_4^{+} + SO_4^{2-} \quad (2)$$

$$SO_4^{+} + APAP \rightarrow RO^* + SO_4^{2-} \quad (3)$$

The excitation of $K_2S_2O_8$ at 266 nm in presence of APAP leads to the transformation of the initial spectrum of $SO_4^{+}$ radical ($\lambda_{\text{max}} = 455$ nm [25]) to the transient spectrum of RO* (Fig. 2a) with maxima at 320 and 445 nm [8]. This spectrum is in agreement with the one obtained in direct photolysis of APAP [19], which proves the formation of RO* upon laser excitation at 266 nm. The rate constant ($k_3 = 6.7 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$) of reaction (3) was calculated from the linear dependence of the observed rate constant of RO* radical formation at 320 nm from the concentration of APAP (Fig. 2c).

value is close to the diffusion rate constant and to the rate constant of hydroxyl radical reaction with APAP ($9.8 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$ [8]).

In our previous paper practically linear dependence of both hydrated electron and RO* at direct 266 nm photolysis of APAP was observed leading to conclusion that photoionization is monophotonic process mainly [19]. But more accurate measurements in wider range of excitation energy gives slope 1.6 for the yields of species on laser excitation energy in log–log coordinate (Fig. 3). This is clear evidence that two-photon process play the important role in APAP photoionization under laser excitation. The cut-off on the ordinate (Fig. 3, insert) allows to estimate the quantum yield of monophotonic process ($\psi_{\text{mono}} \approx 10^{-2}$).

3.2. Stationary photolysis of APAP and P1

Irradiation at 254 nm leads to APAP disappearance with quantum yield $\psi_{254} = 1.4 \times 10^{-3}$ and to formation of single main primary product which was identified by optical spectra and LC–MS as photo-Fries product P1 (retention time 12.5 min, $m/z$ 152.071, $C_6H_{10}NO_2$, Table 1 and Fig. 4b and c) [16,17]. The formation of one

![Fig. 2](image_url)

Fig. 2. (a) Transient absorption spectra recorded 0.05 (1), 0.4 (2), 1.6 (3), 4 (4) and 9.6 (5) $\mu$s after the laser excitation of $K_2S_2O_8$ (0.04 M) in the presence of APAP ($6.5 \times 10^{-3}$ M). (b) Characteristic kinetic curves at 320 and 450 nm. (c) The dependence of the observed rate constant of RO* radical formation at 320 nm on concentration of APAP.

![Fig. 3](image_url)

Fig. 3. (a) The dependence of the hydrated electron ($\lambda_{\text{reg}} = 720$ nm, argon-saturated solutions) and RO* ($\lambda_{\text{reg}} = 440$ nm, air-equilibrated solutions) yields on laser excitation energy in log–log coordinate. The slope of both lines is 1.6. (b) The dependence of the observed quantum yield of the hydrated electron on excitation energy.

![Fig. 4](image_url)

Fig. 4. Optical spectra (a) of air-saturated APAP ($8.3 \times 10^{-3}$ M) solution after 0 (1), 7 (2), 20 (3), 30 (4), 40 (5) and 200 (6) min of irradiation at 254 nm. The base peak (b) and UV (c) chromatograms after 0 (1), 20 (2) and 200 (3) min of irradiation, accordingly. *Impurity in the eluent. The energy of excitation was $6 \times 10^{-4} \text{ E} \cdot \text{min}^{-1}$.
photoprodut is also confirmed by conservation of isoosbestic points at 239 and 262 nm during initial stages of photolysis (Fig. 4a). The quantum yield of APAP disappearance at 254 nm is rather close to value 10^{-3} published in Martignac’s work [17] and is the same order of magnitude as was found for acetanilide (6.6 × 10^{-3} [26]).

Except P1 another minor product with retention time 7.1 min was determined as aminophenol [P2, m/z 110.06, C6H10NO, Fig. 4b] in agreement with Martignac’s work [17]. Aminophenol is more likely a product of bulk reactions of primary intermediate (aminyl radical) escaped the geminate recombination (reaction (1)).

The prolonged irradiation at 254 nm for 200 min leads to 95% of APAP disappearance and the predominant formation of two secondary products P5, (m/z 168.065, C6H10NO2) and P4 (m/z 184.060, C6H10NO4) (Fig. 4b, c and Table 1). P4 is formed most probably due to secondary photolysis of photogenerated P1, as P4 was found to be the product of P1 photolysis (Fig. 7b). P4 was also observed as a product of prolonged APAP photolysis in Martignac’s work [17]. It was assumed that P4 is peroxyester R-C(O)-OOCH3 formed by consequence of reactions including the C=C bond photocleavage, the oxygen attachment to CH3 radical and the recombination of radical species [17]:

\[ R-C(O)-CH_3(P1) \rightarrow R-C(O)\cdot \cdot \cdot CH_3-O_2 \rightarrow R-C(O)-OOCH_3 \]

However, fragment ions of the parent ion (m/z 184.06) of the P4 were detected at m/z 138.053 (loss of HCO(O)OH), 124.040 (loss of CH2=CH=O and H2O) and 98.057 (loss of CH2=CH=O and HCO(O)OH) (Table 1S and Fig. 2S, ESI). No fragment ions with characteristic mass corresponding to loss of CH3O, CH3OO or C(O)-OOCH3
groups were detected. So we assumed that P4 is not the peroxyster but a ring-opened product (2E,4Z)-2-acetyl-3-amino-6-oxohexa-2,4-dienoic acid formed by oxidation of phenoxyl radicals derived upon P1 photoionization by O2•−. This assumption has to be proved by laser flash photolysis of P1. The mechanism of P5 formation will be discussed later. Also two minor products (P3 and P6) of APAP prolonged photolysis at 254 nm (Table 1) were detected by LC–MS. Both P3 (m/z 274.070, C14H12NO4) and P6 (m/z 222.076, C11H12NO4) exhibit similar fragment ions with characteristic mass losses of CH3=CH=O and several H2O and CO molecules (Table 1S, Figs. 1S and 5S, ESI). P6 was tentatively assigned to 3-(3-acetyl-2-amino-5-hydroxyphenyl)-2-oxopropanal, the product of P1 reaction with the fragment of oxidized aromatic ring of APAP (Fig. 6S and Table 1S, ESI). Unfortunately the existing MS/MS data for P3 (Fig. 1S and Table 1S, ESI) could be assigned to several possible chemical structures and the exact identification of P3 seems to be impossible.

The excitation at 282 nm leads to APAP disappearance with the quantum yield φ282 = 1.6 × 10−3 which is equal to φ254 in the range of experimental error. The same main product P1 and minor product P2 as at 254 nm excitation were observed but also products with retention time 11.1 and 11.4 min (P4 and P5) were formed (Fig. 5b and c). P5 product clearly appears at early stage of photolysis when concentration of P1 is negligible so its formation is not connected to P1 photolysis and is due to photochemistry of APAP itself. It is worth to note that 282 nm excitation leads to faster decay of aromatic products (monitored by absorption at 250 nm) in comparison to 254 nm photolysis. The practically complete disappearance of aromatic products was observed after 280 min of irradiation at 282 nm (Fig. 5b, curve 3) though many peaks still present after 200 min of irradiation at 254 nm (Fig. 4b, curve 3).

The laser excitation at 266 nm (where two-photon ionization is the main process) leads to APAP disappearance with the quantum yield 0.013 which is practically one order higher than was observed at 254 or 282 nm excitation. The hydroxylated APAP, P5 (m/z 168.066, C16H10NO4, Table 1, Figs. 6b and 3S, ESI) is observed as the single main photoproduct. The formation of one predominant photoproduct is also confirmed by conservation of isosbestic points at 227 and 268 nm during initial stages of photolysis (Fig. 6a). This product most probably is formed by disproportionation of two phenoxyl radicals or reaction of phenoxyl radical with the superoxide radical anion (the product of the hydrated electron reaction with dissolved oxygen) [19]. The photo-Fries product is also formed (Fig. 6b and c) but as the quantum yield of photo-Fries reaction is ≈1.5 × 10−3, P1 is only minor product in these conditions.

The assignment of P5 to the product of APAP photoionization gives evidence that such process plays role in APAP photochemistry under 282 nm light excitation too. It is worth to note that quantum yield of photo-Fries reaction at 282 nm is lower in comparison to 254 nm excitation as the overall quantum yield of APAP disappearance is practically the same at both wavelengths and the ratio of P5:P1 is greatly increase at 282 nm photolysis.

Photolysis of synthesized P1 product at 282 nm was also done in order to clarify nature and mechanism of APAP photoproducts formation. At the initial stages of P1 photolysis no good isosbestic points and the formation of several photoproducts were observed (Fig. 7). One of the main photoproduct is the same as to be found at photolysis of APAP at 254 and 282 nm (P4, m/z 184.060, C8H10NO3, Table 1). Other primary photoproducts of P1 photolysis with retention time 18.0 (S1, m/z 287.141, C16H12N2O2) and 21.5 min (S2, m/z.

288.124, C_{16}H_{18}NO_{4}) were not observed at APAP photolysis (Table 1S, Fig. 7S and 8S, ESI). These dimeric products exhibit similar fragmentation picture and most probably are formed in reactions of some reactive intermediates with P1 itself, as its concentration (10^{-4} M) was one order higher as one found in APAP photolysis experiments (10^{-5} M). For this reason the detailed identification of the structures of S1 and S2 was not done in this work.

3.3. Mechanism of APAP photolysis in the different experimental conditions

The results of steady-state and flash photolysis experiments allow us to make several conclusions concerning APAP photochemistry in aqueous solutions:

1. Laser excitation at 266 nm leads to predominant two-photon ionization of APAP with the formation of N-(3,4-dihydroxyphenyl)acetamide (hydroxylated APAP, P5) as the main photoproduct. The monophotonic ionization also takes place with the quantum yield about 10^{-3}.

2. Steady state photolysis of APAP at 254 nm leads to the formation of 1-(2-amino-5-hydroxyphenyl)ethanone (P1) as the main primary photo-Fries product (reaction (1)). P5 photoproduct was detected at the prolonged irradiation of APAP at 254 nm and was not observed at steady-state photolysis of P1.

3. Steady state photolysis of APAP at 282 nm leads to the formation both P1 and P5 as the primary photoproducts clearly indicating the competition of two photochemical channels – photo-Fries reaction (P1) and monophotonic ionization (P5).

4. Prolonged UV irradiation of APAP at 254 nm and P1 at 282 nm leads mainly to P4 product formation.

5. The increase of photoionization quantum yield and the corresponding decrease of the photo-Fries reaction yield with increasing of excitation wavelength was observed.

6. Excitation at 282 nm is more favorable to the degradation of aromatic ring of APAP and its photoproducts in comparison to 254 nm excitation.

Based on aforesaid conclusions the following scheme of APAP photolysis was proposed (Fig. 8). Two different photochemical channels are reflecting dual nature of APAP molecule. On the one hand it exhibits properties of substituted acetanilides and undergoes photo-Fries reaction [17,18]. On the other hand APAP could be treated as substituted phenol and undergoes the photoionization in aqueous solution which is a typical process for phenols [22,27].

Most probably, the wavelength-dependent photochemistry of APAP could be explained by population of different excited states of molecule upon irradiation. Indeed, the UV absorption spectrum of APAP is typical for substituted acetanilides and consists of two overlapping bands assigned to S_0 → S_2 (243 nm) and S_0 → S_1 (ππ^*) (280 nm) transitions (Fig. 1) [28,29]. It is known that photo-Fries reaction takes place in the picosecond time scale from the dissociative ππ^* state populated by internal conversion from the lowest S_1 (ππ^*) state [18,30]. Probably excess of excitation energy is favorable to the population of the dissociative ππ^* state. In order to get deeper insight into the photochemistry of APAP one need to use the quantum chemical calculations.

4. Conclusions

In this work, mechanistic aspects of acetaminophen photochemistry in aqueous solution were investigated by combination of laser flash and steady-state photolysis with HPLC and LC-MS. Two competitive primary photoproducts – photo-Fries reaction and photoionization were observed. The first processes dominates at short-wavelength (254 nm) excitation and leads to the formation of 1-(2-amino-5-hydroxyphenyl)ethanone (P1) as the main primary photo-Fries product. The photoionization is more important at long-wavelength excitation (282 nm) and leads to the formation of the single main product of phenoxyl radical reactions – N-(3,4-dihydroxyphenyl)acetamide (P5). Both photoproducts have rather low quantum yields (about 10^{-3}). It was found that excitation at 282 nm is more favorable to degradation of aromatic ring of APAP and its photoproducts in comparison to 254 nm excitation. Nature of both primary and secondary photoproducts of APAP photolysis in different conditions was determined by LC–MS and the mechanism of wavelength-dependent APAP photochemistry was proposed and discussed. The results clearly show that both wavelength and light intensity could significantly influence on the phototransformation of APAP in aqueous solutions. The findings of this work also indicate that excimer UV lamps exhibit better performance to both APAP and its aromatic photoproducts removal than low-pressure Hg lamps.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jphotochem.2013.10.006.

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