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Degradation of caffeine and identification of the transformation products generated by ozonation

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Abstract

The ozonation of caffeine in water was performed at different pH values, including acidic conditions. Kinetic experiments were conducted by adding pulses of a concentrated caffeine solution to ozone saturated water. The results showed a rapid decrease of ozone concentration during the first 15 s after injection, followed by a gradual decline at a much slower rate. The data were fitted to a second order kinetic model with rate constants increasing from 0.25 to 1.05 $M^{-1}s^{-1}$ for pH in the 3-10 range. The initial ozone consumption per mole of ozonated caffeine was greater at high pH values, reflecting a higher ozone decomposition rate. The decomposition of ozone was positively affected by the concentration of caffeine, an effect that could be attributed to the presence of a reaction intermediate from the ozonation of caffeine that behaved as a strong promoter of ozone decomposition. A study of the transformation products identified by LC-TOF-MS was carried out, which permitted a tentative degradation pathway to be proposed persistent by-products to be identified at both pH 3 and 8. Most transformation products were the result of the opening of the imidazole ring after breaking caffeine's N7C8 double bond.

Keywords: Caffeine; ozonation; mineralization; oxidation intermediates

1. Introduction

The growing scarcity of water resources is one of the most critical environmental problems that need to be solved in the near future. The use of reclaimed water has been highlighted as playing an important role in the management of water resources. Generalized reuse of wastewater will reduce the consumption of drinking water and allow water pollution to be controlled in rivers and groundwaters, especially in arid and semi-arid regions which suffer periodic acute water shortages. Reclaimed wastewater may find several applications such as, for example, the irrigation of agricultural and urban areas, cooling water for industrial plants and recharging groundwater bodies (Petalaa et al., 2006). A major hurdle to this endeavour is the evidence that the conventional treatment of municipal wastewaters discharges many biologically-active compounds into the environment. The improvement of analytical techniques has enabled the detection of a number of compounds not routinely analysed in the past, not only in the effluent of sewage treatment plants (STP), but in many surface streams and groundwaters. Most of them belong to the group of emerging pollutants, which are candidates for future regulation based on their potential effects on human health and on the environment (Gagné et al., 2006). Ternes et al. (1998) identified over thirty drugs in the influent of German Municipal Wastewater Treatment Plants. In their wake, many studies have revealed the presence of drugs, disinfectants, and other active substances in the environment (Kümmerer, 2001; Tauxe-Wuersch et al., 2005). The need for new treatment strategies is made more acute by the fact that many of these pollutants escape to conventional wastewater treatments with the risk of becoming ubiquitous (Carballa et al., 2004).

Caffeine is by far, the most commonly used legal drug in the world either in the form of beverages or in combination with analgesics to enhance their effect. Caffeine is widely metabolized by humans, the disposal of the unconsumed coffee and caffeinated soft drinks being the predominant source of caffeine introduced into the wastewater treatment system (Tang-Liu et al., 1983). As a result, caffeine has been detected in many surface streams and STP effluents (Franke et al., 1995; Ternes et al., 2001; Kolpin et al., 2002). Lee and Rasmussen (2006) reported the presence of caffeine in samples collected upstream of STP discharges, as well as persistent concentrations downstream. This distribution pattern, different from other organic pollutants, is probably linked to the existence of additional sources such as unauthorized discharges. Caffeine was also detected in the effluent of the municipal STP located in Alcalá de Henares at a level of 600 ng/L (Rodríguez et al., in press). Once discharged, caffeine persists in the environment because of its high solubility in water (37.47±1.01 g/L according to Sriamornsak and Kennedy, 2007) and low octanol-water partition coefficient (Gossset et al., 1983). These facts, together with the lack of biogenic sources, led to caffeine being proposed as a tracer of domestic pollutants (Seiler et al., 1999).

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Ozone has been used extensively for disinfection or to remove dissolved organic matter from surface water, groundwater, and wastewater. When used in alkaline conditions, the decomposition of ozone produces a considerable amount of hydroxyl radicals and falls into the group of Advanced Oxidation Processes. AOP which make use of ozone are effective in the mineralization of organic compounds with a substantial lowering of the COD or TOC of many effluents (Rodríguez et al., in press). However, in some cases the formation of toxic oxidation by-products has been reported (Fernández-Alba et al., 2002). Carr and Baird (2000) studied the ozonation of caffeine at pH 3 with or without the addition of hydrogen peroxide. They found a bimodal mineralization pattern that which they attributed to the formation of partially oxidized intermediates that undergo further oxidation. Kolonko et al. (1979), Stadler et al. (1996), Telo et al., (1997) and Dalmazio et al., (2005) also studied the oxidation of caffeine and proposed some reaction intermediates for the hydroxyl mediated reaction. This work evaluates the ability of ozonation to oxidize caffeine at different pH values including acidic conditions at which the concentration of hydroxyl radicals is low, explores the kinetics of ozone-caffeine reaction and identifies some reaction intermediates.

2. Experimental

2.1. Materials and ozonation procedure

Caffeine was supplied by Sigma-Aldrich (99% purity). Reaction runs were carried out in a 5-L glass jacketed reactor whose temperature was controlled by a Huber Polystat cc2 thermostatic regulator. The temperature of the liquid inside the reactor was monitored throughout the experiment by means of a Pt100 RTD. Ozone was produced by a corona discharge ozonator (Ozomatic, SWO100) fed by oxygen (about 95% purity) produced by an AirSep AS-12 PSA oxygen generation unit. A mixture of ozone and oxygen was bubbled into the liquid by means of a porous glass disk with a gas flow of 0.20 Nm^3/h. At a prescribed time, a pulse of concentrated caffeine was introduced inside the reactor and the gas flow was stopped. Some additional experiments were performed with a continuous gas flow throughout the run. The reaction vessel was agitated at a rate of 500 rpm using a four-blade turbine. Details are given elsewhere (Rosal et al., in press). The initial ozone-to-caffeine molar ration varied in a 0.6-8 range. The experiments were carried out at pH in the 3-10 range, controlled by pumping a diluted sodium hydroxide using a feed-back PID control. During the run, certain samples were withdrawn at prescribed intervals. Ozone was quenched in samples by adding a concentrated solution of sodium thiosulfate or by bubbling nitrogen.

2.2. Analysis

The concentration of dissolved ozone was determined by means of a Rosemount 499A OZ amperometric analyser equipped with Pt 100 RTD temperature compensation and calibrated against the Indigo Colorimetric Method (SM 4500-O_3 B). The signal was transmitted via a Rosemount 1055 SoluComp II Analyser to a Agilent 34970 Data Acquisition Unit and recorded. The concentration of ozone in the gas phase was determined with a non-dispersive UV Photometer Anseros Ozomat GM6000 Pro calibrated and tested against a chemical method. The temperature inside the reactor was measured with a Pt100 RTD. pH was measured with a CRISON electrode connected to a Eutech αlpha-pH100 PID control device that delivered a solution of sodium hydroxide by means of a LC10AS Shimadzu chromatography pump. The system allowed pH to be controlled within ±0.1 units throughout the experiment. The signals from temperature and pH sensors were also sent to the Data Acquisition Unit and recorded in a computer. Total organic carbon (TOC) analyses were carried out by means of a Shimadzu TOC-VCSH total carbon organic analyzer equipped with an ASI-V autosampler.

The identification of caffeine transformation products was carried out by liquid chromatography coupled to time-of-flight mass spectrometry (LC-TOF-MS). The Agilent Series 1100 liquid chromatograph (Agilent Technologies, Palo Alto, USA) was equipped with a reversed-phase C18 analytical column of 3 mm x 250 mm, 5 um particle size (ZORBAX, SB-C18, Agilent Technologies). The mobile phase was a mixture of 0.1% formic acid and 5% Milli-Q water in acetonitrile as mobile phase A and 0.1% formic acid in water as mobile phase B (pH 3.5) at a flow rate of 0.4 mL min^{-1}. A linear gradient progressed from 10%A (initial conditions) to 100%A in 30 min, and then remained steady at 100%A for 5 min. The injection volume was 20 μL. This HPLC system was connected to a TOF mass spectrometer (Agilent Technologies) equipped with an electrospray interface operating under the following conditions: capillary 4000 V, nebulizer 40 psig, drying gas 9 L min^{-1}, gas temperature, 300ºC; skimmer voltage, 60 V; octapole dc1, 37.5 V; octapole rf, 250 V. Routine analyses of caffeine were performed by HPLC using a Hewlett Packard apparatus equipped with a Kromasil C18 250 x 4.6 mm column. The mobile phase was a mixture of acetonitrile and water (75:25) adjusted to pH 2.5 using orthophosphoric acid with an isocratic flow of 0.5 mL/min at room temperature. Injections were performed with a 20 μL loop and UV detection was carried out at 273 nm.

3. Results and discussion

3.1. Kinetics of ozonation

The kinetic data corresponding to the ozonation of caffeine were taken in runs in which the ozone gas flow was stopped immediately after the injection of caffeine. Fig. 1 shows, the evolution of the concentration of caffeine and ozone over time for runs performed at pH 3-10. The concentration of dissolved ozone was monitored with a sampling period of 5 s throughout the run and in all cases showed a two-stage pattern consisting of an initial rapid decline followed by a second period with a lower reaction rate. The difference in reaction between both periods was more pronounced for runs performed at lower pH and can be associated with the rapid depletion of caffeine that took place during the first part of the runs. Caffeine reacted quickly within the initial 15 s period before subsequently declining at a much slower rate, a pattern that suggested the existence of an intermediate compound whose oxidation was the rate controlling step. Table 1 shows some results of the ozonation of caffeine for runs performed at different pH and initial ozone-to-caffeine molar ratio. The rate of ozone depletion was in all cases greater for runs performed at higher pH values, as expected due to the role of hydroxide anion in the ozone decomposition reaction (Tomiyasu et al., 1985; Hoigné, 1998). Thus, the moles of ozone consumed per mole of caffeine ozonated during the first 15 s increased from 0.5 to 3.8 by increasing pH from 3 to 10. The conversion of caffeine during the first 15 s decreased in parallel from 0.61 to 0.11 for increasing pH, a result that was probably linked to the reduction of ozone exposure originated by the increased ozone decomposition rate under alkaline conditions.

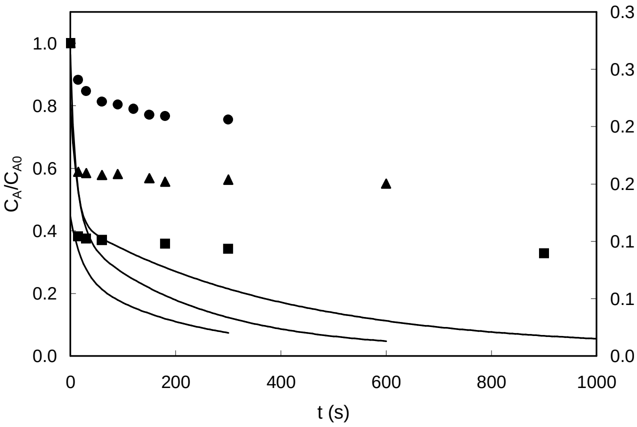

Figure 1. Evolution of the concentration of caffeine for runs performed at pH 3 (■), 5 (▲) and 10 (●). Solid lines and right scale correspond to the ozone profile for the same runs.

The ozonation of a given compound in water is a consequence of the combination of direct and indirect or radical oxidation reactions. Assuming second order rate expressions for the direct reaction of ozone with the organic compound, its rate of disappearance is:

$$-\frac{d\,C_A}{d\,t} = k_{O_3}\,C_A\,C_{O_3} + k_{HO\bullet}\,C_A\,C_{HO\bullet} \qquad [1]$$

The kinetic constants k_{O_3} and $k_{HO\bullet}$ represent the direct and indirect ozone reactions respectively. The integration of Eq. 1 requires information about the concentration of hydroxyl radical that is generally so low that it becomes very difficult to measures directly. Elovitz and coworkers (Elovitz and von Gunten, 1999; Elovitz et al., 2000) proposed a kinetic approach based on a parameter R_{ct} defined as the relationship between the integral *ct-exposure* to ozone and to the hydroxyl radical, the two oxidant species involved in the system:

$$R_{ct} = \frac{\int C_{HO\bullet}\,dt}{\int C_{O_3}\,dt} \qquad [2]$$

The combination of Eqs. 1 and 2 yields, for the ozonation of a given organic compound with initial concentration C_{Ao}, the following expression in which the independent variable is integral ozone exposure:

$$\ln\frac{C_{Ao}}{C_A} = k_{O_3}\int_0^t C_{O_3}dt + k_{HO\bullet}\int_0^t C_{HO\bullet}dt = \left(k_{O_3} + R_{ct}k_{HO\bullet}\right)\int_0^t C_{O_3}dt = k_R\int_0^t C_{O_3}dt \qquad [3]$$

Fig. 2 shows the logarithmic decline of caffeine (A) as a function of integral ozone exposure for runs performed at pH 3-10 and with two different initial ozone-to-caffeine molar ratios. During the first period (< 15 s), a rapid reaction took place in all runs followed by a much slower decline in caffeine concentration. The second slow reaction was first order in the concentration of dissolved ozone. The fitting of experimental results to Eq. 3 permitted kinetic constants to be calculated for the ozonation reaction, k_R. The results shown in Table 1 indicated that the ozonation rate was higher in alkaline conditions due to the greater concentration of hydroxyl radicals associated with the reaction of ozone with hydroxide anion (Beltrán, 2004). On the other hand, the initial conversion of caffeine was a function of pH and decreased from 60% (pH 3) to about 10% (pH 10) as pH increased (Table 1).

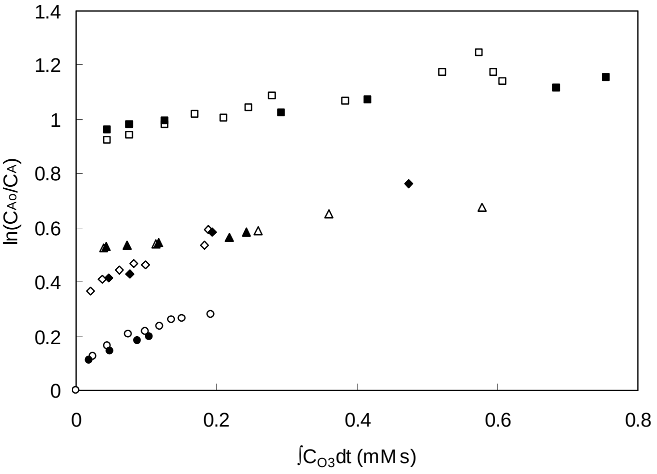

Figure 2. Ozone exposure plot for the ozonation of caffeine for different ozone-to-caffeine initial molar ratios. pH 3 with molar ratios 1.07 (□) and 0.70 (■); pH 5 with ratios 1.01 (Δ) and 0.79 (▲), pH 8 and ratios 0.94 (◇) and 0.78 (◆) and pH 10 at ratios 1.05 (□) and 0.75 (●).

The reduction of TOC was low irrespective of the pH of runs. Fig. 3 shows the profiles of caffeine, ozone, and TOC during an ozonation in which the ozone gas flow was maintained throughout the run. In this case, caffeine became depleted at about 30 min, a period in which the concentration of dissolved ozone was low and increased steadily thereafter. For this run, the extent of mineralization was less than 10% after 120 min, but it remained low even for runs performed under alkaline conditions. For example, TOC decline was 17 % at 120

Table 1. Relative ozone consumption and caffeine conversion (x_A) during the first 15 s of ozonation, Second order reaction constant of caffeine (k_R) and first order rate constant for the decomposition of ozone in pure water (k_{dW}).

pH	$\Delta N_{O3}/\Delta N_A$ (15 s)	k_R ($M^{-1}\ s^{-1}$)	x_A (15 s)	k_{dw} (s^{-1})
3	0.49	0.251	0.61	4.1×10^{-4}
5	0.97	0.306	0.40	8.3×10^{-4}
8	1.23	0.824	0.29	6.1×10^{-3}
10	3.76	1.05	0.11	3.2×10^{-2}

min in a run performed at pH 8 under constant ozone gas flow. In runs where gas flow was stopped, the reduction of TOC was always less than 4% in 120 min. These results pointed to a rapid initial direct reaction between caffeine and molecular ozone, probably an attack on the N7=C8 double bond whose products kept the concentration of dissolved ozone low as long as caffeine existed in solution. The reaction rate of caffeine was low even at pH 10 at which the concentration of hydroxyl radicals should be higher.

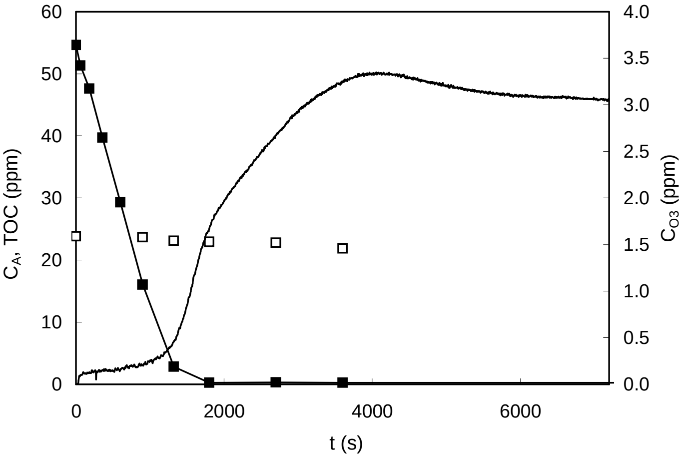

Figure 3. Concentration of caffeine (■) and TOC (□) during an ozonation run with continuous flow of ozone at pH 3, 25ºC and $C_{O3(g)}$ = 12 g/Nm^3. Solid line and right scale show the concentration of dissolved ozone.

The decomposition of ozone after the injection of caffeine exhibited first-order kinetics in two stages. A quick initial reaction was followed by a second period with a lower rate constant, the transition being clearer for runs performed at low pH. The transition was associated with the end of the initial rapid consumption of caffeine, suggesting that the decomposition of ozone was the consequence of two parallel processes, one of which involves a reactive intermediate produced from caffeine:

$$A \xrightarrow{k_R} I \xrightarrow{k_{R,I}} \text{Products}$$

Assuming the steady-state hypothesis, the concentration of this intermediate must be proportional to that of caffeine and the mass balance to ozone in solution yields:

$$-\frac{d\,C_{O_3}}{d\,t} = k_{d,0}\,C_{O_3} + \frac{k_{d,1}\,k_R}{k_{R,I}} C_{O_3}\,C_A \qquad [4]$$

The reaction constant $k_{d,0}$ is a pseudo-first order rate constant associated with the matrix effect. The combination of the differential form of Eq. 3 and Eq. 4, yields a functional form for the concentration of ozone as a function of time:

$$-\frac{d\,C_{O_3}}{d\,t} = k_{d,0}\,C_{O_3} + \frac{k_{d,1}\,k_R}{k_{R,I}} C_{O_3}\,C_{Ao}\,e^{-k_R \int C_{O_3}\,dt} \qquad [5]$$

At the beginning of the run, for low reaction time, the exponential term still approached unity, so that the apparent first-order constant for the decomposition of ozone should be linear with the initial concentration of caffeine:

$$k_d\,(t \to 0) = k_{d,0} + \frac{k_{d,1}\,k_R}{k_{R,I}} C_{Ao} \qquad [6]$$

Fig. 4 shows the apparent rate constant k_d as a function of the initial concentration of caffeine. Results reasonably followed Eq. 6 for pH in the 3-8 range with an intercept of 0.03 s^{-1} representing the matrix effect on the decomposition of ozone free of reactive intermediates produced from caffeine. The slope was 0.75 L mmol^{-1} s^{-1}, a low value which probably reflects a high reaction constant $k_{R,I}$.

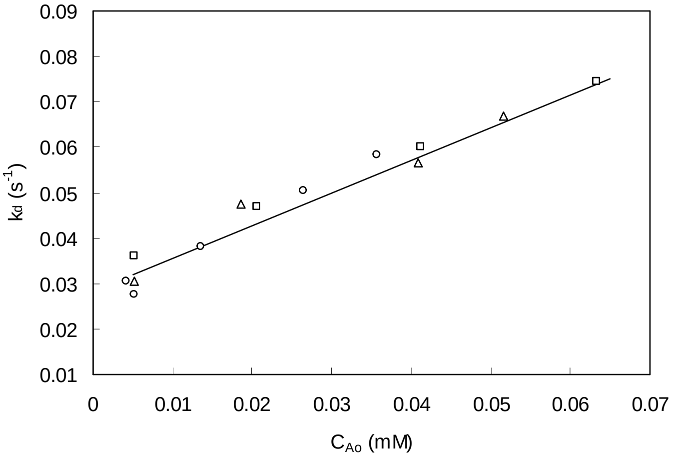

Figure 4. Apparent first-order constant for the decomposition of ozone during the first part of runs at pH 3 (□), pH 5 (Δ) and pH 8 (□).

3.2. Identification of ozonation products

The TOC measurements performed during the ozonation experiments indicated a low extent of mineralization even in conditions at which caffeine became completely depleted (Fig. 3). The organic compounds originated during the ozonation of caffeine largely remained in solution and, consequently, the process is likely to originate persistent organic intermediates which are necessarily less oxidizable than caffeine. There is some information available on reaction products from the advanced oxidation of caffeine in different conditions. Dalmazio et al. (2005) reported reaction intermediates of

Table 2. Accurate mass measurements obtained by LC-TOF-MS for Caffeine (A) and its identified ozonation products at pH 3 and 8.

Compound	Formula	Experimental mass (m/z)	Calculated mass (m/z)	Error (ppm)	DBE
pH = 3					
A	$C_8H_{11}N_4O_2$	195.0876	195.0875	0.27	5.5
P1	$C_8H_{11}N_4O_5$	243.0724	243.0723	0.02	5.5
	$C_8H_{10}N_4O_5Na$	265.0547	265.0543	1.35	5.5
	$C_8H_9N_4O_4$	225.0620	225.0618	0.75	6.5
P2	$C_8H_{12}N_4O_4Na$	251.0751	251.0750	0.09	4.5
	$C_7H_{10}N_3O_3$	184.0713	184.0716	-1.99	4.5
	$C_5H_7N_2O_2$	127.0499	127.0502	-2.37	3.5
P3	$C_7H_{11}N_4O_3$	199.0824	199.0825	-0.84	4.5
	$C_5H_8N_3O_2$	142.0609	142.0611	-1.43	3.5
P4	$C_5H_8N_2O_3Na$	167.0426	167.0427	-0.67	2.5
	$C_3H_9N_2O$	89.0713	89.0709	4.04	0.5
pH = 8					
A	$C_8H_{11}N_4O_2$	195.0876	195.0875	-0.78	5.5
P5	$C_6H_9N_3O_4Na$	210.0485	210.0484	0.60	3.5
	$C_6H_8N_3O_3$	170.0560	170.0560	-0.10	4.5
P6	$C_5H_8N_3O_3$	158.0560	158.0558	-1.37	3.5
P7	$C_8H_{11}N_4O_4$	227.0774	227.0774	-0.35	5.5
	$C_6H_8N_3O_3$	170.0561	170.0560	0.48	4.5
	$C_5H_8N_3O_2$	142.0613	142.0611	1.38	3.5

caffeine under the oxidative conditions of the H_2O_2/UV, TiO_2/UV, and Fenton systems. They state that the initial attack of hydroxyl radicals on the C4=C5 double bond resulted in the formation of *N*,*N*-dimethylparabanic acid further oxidized to bis(*N*-hydroxymethyl)parabanic acid. It has also been reported that the oxidation of caffeine by hydroxyl radicals yielded 1,3,7-trimethyluric acid, from hydroxylation on C8, and theophyline and other N-demethylated products, such as 1,3-dimethyluric acid (Telo et al., 1997). Stadler et al. (1996) identified 6-amino-5-(*N*-formylmethyl-amino)-1,3-dimethyl-uracil, a product resulting from the opening of the imidazole ring. In this work, the identification of caffeine oxidation by-products was based on the accurate mass measurements recorded by a time-of-flight mass spectrometric instrument. Such measurements permit elemental compositions to be proposed for the molecular ions of the detected compounds as well as of their characteristic fragment ions and sodium adducts, thus providing a high grade of confidence in the structure assignation. Table 2 shows the measured and calculated mass of the protonated ions, the error between them and the proposed empirical formula corresponding to the compounds identified by LC-TOF-MS. Accurate mass measurements recorded for the protonated caffeine molecule (*m/z* 195.0875, $C_8H_{11}N_4O_2$) showed excellent agreement between experimental and calculated m/z values with an error lower than 0.8 ppm. From the experiments of caffeine ozonation at pH 3, four compounds were identified as possible transformation products (see Figure 5a). The compound marked as P1 in Fig. 5 presented an accurate mass of 243.0723, which corresponded to a mass increase of 48 Da with respect to the protonated caffeine molecule. The empirical formula that best matches this experimental mass was $C_8H_{10}N_4O_5$ with an error of 0.42 ppm. Therefore, this compound is likely to be the product of the ring opening of a primary molozonide (P0) resulting from the incorporation of ozone to the N7=C8 double bond of the purine structure. Molozonides are unstable and their ready decomposition leads, in the case of carbon-carbon double bonds, to a carbonyl fragment and a zwitterionic species leading in water to the corresponding carboxyl compound (Cataldo, 2001). The structure proposed for this transformation product was confirmed by the presence of the corresponding sodium adduct at m/z 265.0547 ($C_8H_{10}N_4O_5Na$, 1.35 ppm error) and the fragment ion at m/z 225.0620 Da ($C_8H_8N_4O_4$, 0.75 ppm error), identified as corresponding to the neutral loss of H_2O, which is consistent with the presence of a COOH group and the increase in DBE (double bond equivalency). The loss of oxygen and an increase of two hydrogen atoms, probably by reduction of the N=O group of P1, led to P2, which was confirmed by the presence of the sodium adduct at accurate mass of 251.0750 Da ($C_8H_{12}N_4O_4Na$, 0.09 ppm) and by the reduction of the DBE, as a consequence of the loss of the N=O double bond. The most abundant compound found in deeply ozonated samples, P3, presented an accurate mass of 199.0824 Da ($C_7H_{11}N_4O_3$, -0.84 ppm) and a fragment ion at mass 142.0609 Da, corresponding to the loss of $CONCH_3$ groups of the six member ring. This compound, designated 6-amino-5-(N-formylmethylamino)-1,3.dimethyluracil, had already been identified as a biological metabolite of caffeine

(Schrader et al., 1999). Coeluting at the same retention time as P3, compound P4 at m/z 167.0426 ($C_5H_8N_2O_3Na$, -0.84 ppm) was also detected. As the rest of the transformation product identified, P4 still conserves the six member ring structure but a reduction in the DBE (2.5) suggests the possible disappearance of the double bond and subsequent hydroxylation on C5 or C6.

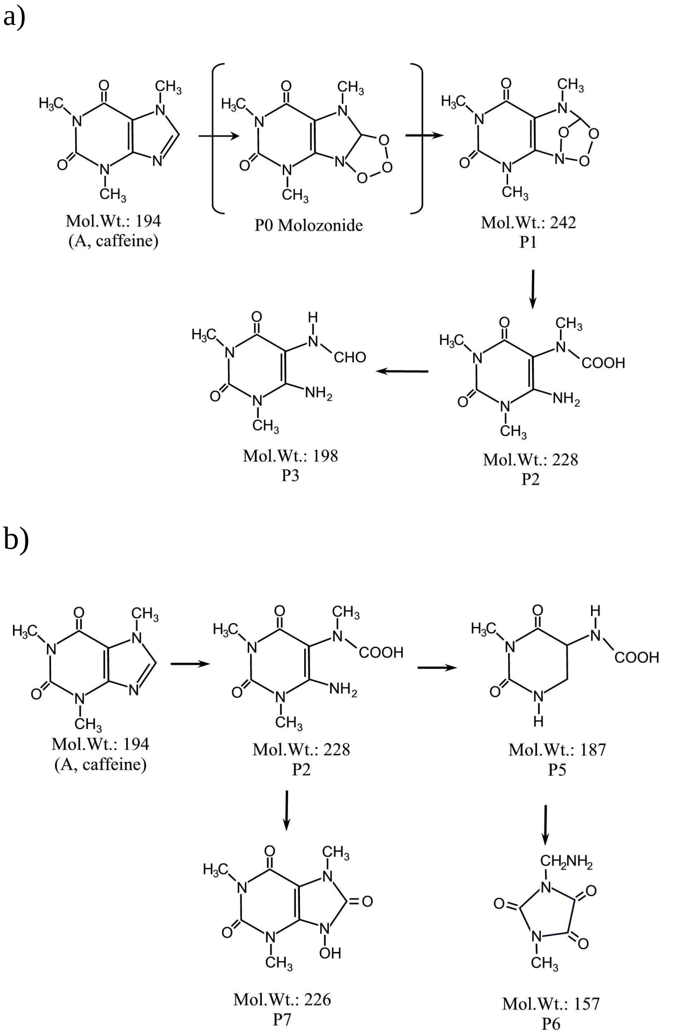

Figure 5. Main products identified by LC-TOF-MS and proposed pathway for the ozonation of caffeine a) in acidic media at pH 3 and b) under alkaline conditions at pH 8.

In ozonation experiments at pH 8, the identified transformation products were assumed to correspond to the oxidation of P2. The accurate mass of 210.0485 Da was assigned to the sodium adduct of P5, $C_6H_9N_3O_4Na$, with an error of 0.60 ppm, and tentatively attributed to the structural formula shown in Fig. 5. Typical neutral loss of H_2O from the carboxylic acid group was also observed at m/z 170.0560. P6, with experimental mass 158.0560 and without significant fragments was assigned to $C_5H_8N_3O_3$, the protonated form of N-methyl-N’-aminomethylparabamic acid, related to the N,N’-dimethylparabamic acid identified by Kolonko et al. (1979) as a by-product of the ozonation of caffeine. P7 was identified with an accurate mass of 227.0774 as ($C_8H_{11}N_4O_4$, -0.35 error) a hydroxylated form of the 1,3,7-trimethyluric acid already identified by Telo et al. (1997). Its fragment ions (170.0561 and 142.0613) correspond to the opening of the imidazole ring and losses of the methyl groups.

4. Conclusions

The ozonation of caffeine was specially rapid during the initial 15 s reaction period, thereafter declining at a much slower rate. This behaviour suggested the existence of an intermediate compound whose oxidation was the rate controlling step. Second order kinetic constants increased from 0.25 to 1.05 $M^{-1}s^{-1}$ for increasing pH in the 3-10 range. The amount of ozone consumed per mole of ozonated caffeine was greater at higher pH values, as was to be expected from the enhanced ozone decomposition at basic pH. As for ozone decomposition, it was also shown to be enhanced when higher concentrations of caffeine were used. This effect could be attributed to the presence of a reaction intermediate behaving as a promoter of ozone decomposition. Irrespective of pH, the reduction of TOC during ozonation was always low, indicating the presence of transformation products. A study of the transformation products identified by LC-TOF-MS allowed us to propose the two different degradation routes for pH 3 and 8, as well as to identify persistent by-products. The results provided evidence that the ozonation of caffeine followed different mechanistic routes depending on the ozonation conditions applied. Most transformation products were the result of the opening of the imidazole ring after N7C8 double bond of caffeine was broken.

Acknowledgements

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References

Beltran, F.J., 2004, Ozone Reaction Kinetics for Water and Wastewater Systems, CRC Press LLC, Florida, 2004, pp. 16-17.

Carballa, M., Omil, F., Lema, J.M., Llompart, M., García-Jares, C., Rodríguez, I., Gómez, M., Ternes, T., 2004, Behaviour of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res., 38, 2918-2926.

Carr, S.A., Baird, R.B., 2000, Mineralization as a mechanism for TOC removal: study of ozone/ozone-peroxide oxidation using FT-IR, Water Res., 34, 4036-4048.

Cataldo, F., 2001, The action of ozone on polymers having unconjugated and cross- or linearly conjugated unsaturation: chemistry and technological aspects, Polym. Degrad. Stab., 73, 511-520.

Dalmázio, I., Santos, L.S., Lopes, R.P., Eberlin, M.N., Augusti, R., 2005, Advanced oxidation of caffeine in water: on-line and real-time monitoring by electrospray ionization mass spectrometry, Environ. Sci. Technol., 39, 5982-5988.

Elovitz, M.S., von Gunten, U., 1999, Hydroxyl radical/ozone ratios during ozonation processes I. The Rct concept, Ozone Sci. Eng., 21, 239–260.

Elovitz, M.S., von Gunten, U., Kaiser, H.P., 2000, Hydroxyl radical/ozone ratios during ozonation processes II. The effect of temperature, pH, alkalinity and DOM properties, Ozone Sci. Eng., 22, 123–150.

Fernandez-Alba, A.R., Hernando, D., Agüera, A., Cáceres, J., Malato, S., 2002, Toxicity assays: a way for evaluating AOPs efficiency, Water Res., 36, 4255–4262.

Franke S, Hildebrandt S, Schwarzbauer J, Link M, Francke W., 1995, Organic compounds as contaminants of the Elbe river and its tributaries Part II: GCyMS screening for contaminants of the Elbe water, Fresenius J. Anal. Chem., 353, 39–49.

Gagné, F., Blaise, C., André, C., 2006, Occurrence of pharmaceutical products in a municipal effluent and toxicity to rainbow trout (Oncorhynchus mykiss) hepatocytes, Ecotox. Environ. Saf., 64, 329-336.

Gossett, R.W., Brown, D.A., Young , D.R., 1983, Predicting the bioaccumulation of organic compounds in marine organisms using octanol/water partition coefficients, Mar. Pollut. Bull., 14, 387–392.

Hoigné, J., 1998, Chemistry of aqueous ozone and transformation of pollutants by ozone and advanced oxidation processes, in The Handbook of Environmental Chemistry, Vol. 5, Part C, Quality and Treatment of Drinking Water II, Springer, Berlin-Heidelberg.

Kolonko, K. J., Shapiro, R.H., Barkley, R.M., Sievers, R.E., 1979, Ozonation of caffeine in aqueous solution, J. Org. Chem., 44, 3769-3778.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002, Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance, Environ. Sci. Technol.,36, 1202-1211.

Kümmerer, K., 2001, Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. Chemosphere, 45, 957-969.

Lee, C.S., Rasmussen, T.J., 2006, Occurrence of organic wastewater compounds in effluent-dominated streams in Northeastern Kansas, Sci. Total Environ., 371, 258–269.

Petalaa, M., Tsiridisa, V., Samarasb, P. Zouboulisc, A., Sakellaropoulosa, G.P., 2006, Wastewater reclamation by advanced treatment of secondary effluents, Desalination, 195, 109–118.

Rodríguez, A., Rosal, R., Perdigón, J.A., Mezcua, M., Agüera, A., Hernando, M.D., Letón, P., Fernández-Alba, A.R., García-Calvo, E., 2008 (in press), Ozone-based Technologies in Water and Wastewater Treatment in D. Barceló and Mira Petrovic (Eds.) Emerging Contaminants from Industrial and Municipal Waste (The Handbook of Environmental Chemistry), Springer-Verlag, Berlín.

Rosal, R. Rodríguez, A, Perdigón-Melón, J.A., Mezcua, M., Hernando, M.D., Letón, P., García-Calvo, E., Agüera, A., Fernández-Alba, A.R., in press, Removal of pharmaceuticals and kinetics of mineralization by O_3/H_2O_2 in a biotreated municipal wastewater, Water Res., in press, doi: 10.1016/j.watres.2008.06.008.

Schrader, E., Klaunicka, G., Jorritsmaa, U., Neuratha, H., Hirsch, K., Kahl , G., Foth, H., 1999, High-performance liquid chromatographic method for simultaneous determination of [1-methyl- ^{14}C]caffeine and its eight major metabolites in rat urine, J. Chrom. B, 726, 195–201.

Seiler, R.L., Zaugg, S.D., Thomas, J.M., Howcroft, D.L., 1995, Caffeine and phamaceuticals indicators of water contamination in wells, Groundwater, 37, 405-410.

Sriamornsak, P., Kennedy, R.A., 2007, Effect of drug solubility on release behavior of calcium polysaccharide gel-coated pellets, Eur. J. Pharm,. Sci., 32, 231–239.

Stadler, R.H., Richoz, J., Turezki, R.J., Welti, D.H., Fay, L.B., 1996, Oxidation of caffeine and related methylxanthines in ascorbate and polyphenol-driven Fenton-type oxidations, Free Radical Res., 24, 225-240.

Tang-Liu, D., Williams R., Riegelman, S., 1983, Disposition of caffeine and its metabolites in man, J. Pharmacol. Exp. Ther. 24, 180–185.

Tauxe-Wuersch, A., De Alencastro, L.F., Grandjean, D., Tarradellas, J., 2005, Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment, Water Res., 39, 1761-1772.

Telo, J.P., Vieira, J.S.C., 1997, Mechanism of free radical oxidation of caffeine in aqueous solution, J. Chem. Soc., Perkin Trans. 2, 1755-1757.

Ternes, T., Bonerz, M., Schmidt, T., 2001, Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography-electrospray tandem mass spectrometry, J. Chromatogr. A, 938, 175-185.

Tomiyasu, H., Fukutomi, H., Gordon, G., 1985, Kinetics and mechanism of ozone decomposition in basic solution, Inorg. Chem., 24, 2962-2966.