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Reactivity of mucohalic acids in water

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ABSTRACT

One group of disinfection byproducts of increasing interest are the halogenated furanones, which are formed in the chlorination of drinking water. Among these halofuranones is mucochloric acid (MCA, 3,4-dichloro-5-hydroxyfuran-2(5*H*)-one), and mucobromic acid (MBA, 3,4-dibromo-5-hydroxyfuran-2(5*H*)-one). Both mucohalic acids (MXA) are direct genotoxins and potential carcinogens, with the capacity to alkylate the DNA bases guanosine, adenosine and cytosine, and they have been measured in concentrations ranging up to 700 ng/l in tap water. MCA and MBA react in basic aqueous medium to form mucoxyhalic acids (4-halo-3,5-hydroxyfuran-2(5*H*)-one). Since: i) this reaction may represent the first step in the abiotic decomposition of mucohalic acids, ii) mucoxyhalic acids have been proposed as possible intermediates in the reaction of MXA with DNA, a kinetic study of the reaction mechanism is of interest. Here, the following conclusions were drawn: a) At moderately basic pH, the reaction of mucohalic acids with OH^- to form mucoxyhalic acids is kinetically significant. b) The nucleophilic attack of hydroxide ions on MXA occurs through a combination of two paths: one of them is first-order in hydroxide whereas the other is second-order and are proposed to occur through the deprotonation of the hydrate of MXA. c) The hydration constants of mucohalic acids −0.23 and 0.17 for MCA and MBA respectively − corresponds to the very significant hydrate concentrations. Since hydrates are not electrophilic, these values imply a decrease in the alkylating capacity of mucohalic acids.

1. Introduction

Disinfection by-products (DBPs) are formed when disinfectants such as chlorine, ozone, chlorine dioxide or chloramines react with naturally occurring organic matter, anthropogenic contaminants, bromide, and iodide during the production of drinking water (Richardson et al., 2007). These DBPs are responsible for most of the observed mutagenicity of chlorinated tap water (Kanniganti et al., 1992; Kronberg et al., 1991, 1988; Meier et al., 1988).

A group of DBPs of increasing interest and yet to be regulated are the halofuranones, which are formed in the chlorination of organic matter (i.e., humic substances), and whose genotoxic and carcinogenic properties are well known (IARC, 2004; McDonald and Komulainen, 2005). Among these halogenated furanones formed in chlorination is mucochloric acid (MCA, 3,4-dichloro-5-hydroxyfuran-2(5*H*)-one), which, like its analogue mucobromic acid (MBA, 3,4-dibromo-5-hydroxyfuran-2(5*H*)-one), is a direct genotoxin and a potential carcinogen (Fekadu et al., 1994; Jansson et al., 1995; Knasmuller et al., 1996; Liviac et al., 2009). Both mucohalic acids (MXA) are also known to alkylate the DNA bases guanosine, adenosine and cytosine, both in the form of monomers and forming part of DNA, giving rise to etheno, oxalo etheno and halopropenal derivatives (Kronberg et al., 1996, 1993, 1992; LeCurieux et al., 1997).

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Mucochloric acid, along with other halogenated furanones, such as the highly mutagenic MX and its chlorinated and brominated analogues, has been detected in drinking waters (plant effluent) at concentrations up to 1 μg/l (Krasner et al., 2006; Kronberg and Franzen, 1993; Kronberg et al., 1988; Smeds et al., 1997). The total concentration of MCA in plant effluent from U.S. water treatment plants ranges from below detection limit (0.02 μg/l) up to 0.71 μg/l, depending on the location of the plant, the season, the amount of natural organic matter and the chlorination product used (Onstad et al., 2008).

Mucohalic acids are known to react in basic aqueous media to form mucoxyhalic acids (MOXA): the α halogen atom of the carboxyl group undergoes nucleophilic substitution by a hydroxide ion (Wasserman and Precopio, 1952, 1954). The direct product of this reaction is the enol tautomer of MOXA ($MOXA_{en}$), which is in tautomeric equilibrium with the keto tautomer ($MOXA_{ket}$), as is shown in Scheme 1.

The reaction of MXA to form MOXA is of ecological importance, since it may represent the first step in a possible abiotic pathway for the degradation of mucohalic acids in the environment. Little is known about the reaction products, mucoxyhalic acids, and their potential environmental and health effects. For instance, mucoxyhalic acids are known to be unstable in acidic conditions (Mowry, 1950; Wasserman and Precopio, 1952) and have been suggested to undergo decarboxylation, yielding highly toxic malondialdehydes (Kronberg et al., 1996, 1993, 1992; LeCurieux et al., 1997). These effects could become significant, should the formation of mucoxyhalic acids be favored under environmentally relevant conditions or during water treatment processes.

In addition, the formation of mucoxyhalic acids has been proposed as a possible initial step in the reaction of MXA with DNA bases (Klika et al., 2006; Kronberg et al., 1996, 1993; LeCurieux et al., 1997). Whereas currently some evidence has discarded mucoxyhalic acids as intermediates in the alkylation reaction of mucohalic acids (Asplund et al., 1995; Mäki et al., 1999), a kinetic study of the formation of MOXA is still lacking.

2. Materials and methods

Mucochloric acid (99%), mucobromic acid (99%) and D_2O were purchased from Aldrich. NaOH (99%) was from Panreac. Water was purified using a MiliQ system.

2.1. Kinetics of MXA decomposition

Reactions were carried in NaOH solutions (pH 11–13.5) that had been previously normalized with potassium hydrogen phthalate. The final concentration of MXA was in the 1×10^{-5}–1×10^{-4} M range and the applied concentration of sodium hydroxide was between 0.01 and 0.50 M for MCA, and 0.005 and 0.10 M for MBA. Reactions were monitored between 15 and 35 °C.

The experimental procedure was as follows: 50 μl of MXA (5×10^{-4}–5×10^{-3} M) in slightly acidic aqueous solution (pH ~4.5) was delivered with a Hamilton syringe to a Hellma quartz UV–Vis cuvette (10.0 mm optical length) containing 3 cm^3 of the thermostated NaOH solution. Immediately after the mixture was complete, data acquisition was started.

Reaction kinetics were monitored at $\lambda = 255$ and 290 nm for MCA and $\lambda = 275$ nm for MBA. UV–Vis spectra and kinetic measurements were carried out on a Shimadzu UV-2401-PC spectrophotometer equipped with a thermoelectric six-cell holder temperature control system (± 0.1 °C). The temperature of the reaction mixtures prior to mixing was kept constant (± 0.05 °C) with a Lauda Ecoline RE120 thermostat.

The UV–Vis, ^1H NMR and mass spectra of the reaction products were consistent with those reported for MOXA in the literature.

2.2. Hydration constants of MXA

The experimental determination of the hydration constants of the two mucohalic acids in neutral aqueous solution was performed as suggested in the literature (Greenzaid et al., 1967; Hooper, 1967). Briefly, solutions of mucohalic acids (0.15 M) were prepared in D_2O, the pH values of these were adjusted to approximately 7.0 by the addition of small amounts of concentrated sodium hydroxide in D_2O, and their ^1H NMR were recorded at 25 °C using a Brucker 400 MHz apparatus.

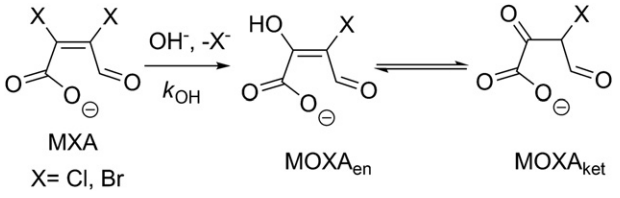
By dividing the area of the peak corresponding to the hydrate ($Area_{MXA\ hyd}$) by the area of the main peak ($Area_{MxA}$), which corresponds to un-hydrated MXA, it is possible to calculate the hydration constants.

$$K_{hyd} = \frac{[MXA\ hyd]}{[MXA]} = \frac{Area_{MXA\ hyd}}{Area_{MXA}} \quad (1)$$

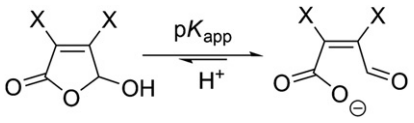
3. Results and discussion

Mucohalic acids are known to exist as an equilibrium between two tautomers: the open-chain aldehyde-acid and the closed-chain lactone-lactol (Scheme 2). In neutral or basic medium, as used in this work, the open-chain form is the most abundant tautomer (Franzen et al., 1999; Mowry, 1950, 1953). The pK_{app} values for this equilibrium (Scheme 2) are 3.95 and 4.27 for MCA and MBA respectively, as measured by optical titration (Franzen et al., 1999).

The reaction products, MOXA, have received only modest attention in the literature, and little is known about their toxicity, stability or tautomeric equilibria. Since the open-chain tautomers of halogenated furanones are strong acids (due to the inductive and conjugative effects of halogen atoms and the double bond, respectively) the species of interest are anionic at the working pH, which hinders the use of the easily available models such as SPARC (SPARC Performs Automated Reasoning in Chemistry) to predict their properties, and *ab initio* or DFT computational approaches are needed.



Scheme 1 – Reaction of Mucohalic Acids with OH^-.



Scheme 2 – Tautomerization of MXA.

Thus, we computed the free energy of $MOCA_{en}$ and $MOCA_{ket}$ in aqueous solution at the DFT B3LYP 6–31++G(2df, 2pd) level of theory with the Polarizable Continuum Model for solvation, using the Gaussian 03 suite of programs (Frisch et al., 2003). The difference in free energy between these two species is less than 1 kJ mol^{-1} ($\Delta G = G_{enol} - G_{keto} = -0.3$ kJ mol^{-1}), which suggests an equilibrium constant very close to unity, and hence, that neither of the two is major and both have similar concentrations in the reaction conditions.

3.1. *Kinetics of MXA decomposition*

The reactions were followed by UV–Vis spectroscopy. In the case of MCA, its disappearance was monitored at $\lambda = 255$ nm, and the appearance of MOCA at $\lambda = 290$ nm (Fig. 1a); equal rate constants were obtained in both cases. In the case of MBA, owing to the low molar absorption of MOBA only the disappearance of MBA was observed ($\lambda = 275$ nm; Fig. 1b).

The following rate equation describes the formation of MOXA from MXA as illustrated in Scheme 1:

$$\frac{d[MOXA]}{dt} = k_{OH}[OH^-][MXA] \quad (2)$$

Since hydroxide ions were present in large excess, their concentration was considered to remain constant, and thus the pseudo-first-order approximation was used, such that $k_{exp} = k_{OH}[OH^-]$, as in eq. (3), and its integrated counterpart, eq. (4).

$$\frac{d[MOXA]}{dt} = k_{exp}[MXA] \quad (3)$$

$$[MOXA] = [MXA]_o\left(1 - e^{-k_{exp}t}\right) \quad (4)$$

Since both MXA and MOXA show significant absorption at the wavelengths of measurement, the total absorbance is

$$\begin{aligned} A &= l[MXA]_o\left(\varepsilon_{MXA}\left(e^{-k_{exp}t}\right) + \varepsilon_{MOXA}\left(1 - e^{-k_{exp}t}\right)\right) \\ &= l[MXA]_o\left(\varepsilon_{MXA} + (\varepsilon_{MOXA} - \varepsilon_{MXA})\left(1 - e^{-k_{exp}t}\right)\right) \end{aligned} \quad (5)$$

$$A = A_o + \Delta A\left(1 - e^{-k_{exp}t}\right) \quad (6)$$

A_o is the initial absorbance and ΔA is the difference in absorption between MXA and MOXA. Fig. 2 shows the excellent fit of the results to eq. (6).

The reaction orders were determined from the slope of the logarithmic plots of $\log k_{exp}$ against $\log [OH^-]$ (Fig. 3). The observed reaction orders with respect to hydroxide ions in the experimental conditions are 1.2 for MCA and 1.8 for MBA. These values suggest that the reaction takes place by both first-order and second-order reaction mechanisms, with rate constants k_1^{OH} and k_2^{OH}, respectively (eq. (7)).

$$k_{exp} = k_1^{OH}[OH^-] + k_2^{OH}[OH^-]^2 \quad (7)$$

Fig. 4 shows the excellent fit of the experimental k_{exp} values to eq. (7). The values obtained for k_1^{OH} and k_2^{OH} are shown in Table 1.

The variation in the rate constants with temperature allows calculation of the activation energies using the Arrhenius equation; the values are shown in Table 2.

The values for k_1^{OH} are somewhat higher for MBA, which together with the lower activation energies is consistent with bromide as the better leaving group. Moreover, k_2^{OH} is almost two hundred times larger in the case of MBA, which can be interpreted in terms of the proposed mechanism (see below and Scheme 3). In general, the high activation energies are consistent with the attack of a hard-charged nucleophile such as the hydroxide ion.

These results show that the reaction of mucohalic acids with hydroxide ions to form mucoxyhalic acids becomes kinetically significant only at moderately high concentrations of OH^-, and hence the reaction rate when the pH value is close to neutrality can be considered negligible. Since the extracellular pH is approximately 7.40 (Ross and Boron, 1981) and pH levels inside human cells are about 7.00–7.40 (Bright et al., 1987) the formation of MOXA from MXA *in vivo* is expected to be negligible. This is in agreement with current evidence suggesting that MOXA do not participate in the alkylation reactions of mucohalic acids (Asplund et al., 1995; Mäki et al., 1999).

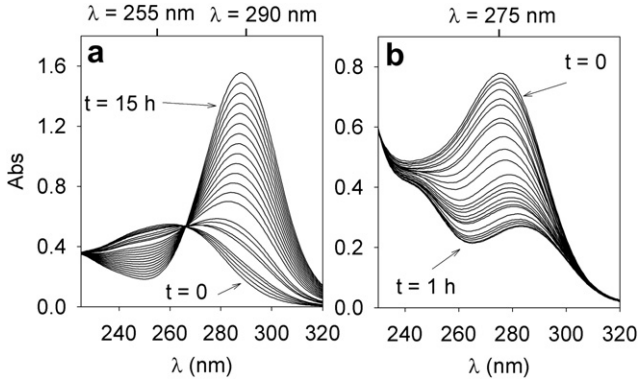


Fig. 1 – Variation with time in the UV–Vis spectra of MXA in the reaction with OH^-; T = 25.0 °C, $[OH^-] = 3.3 \times 10^{-2}$ M; a) X = Cl, [MCA] = 7.5×10^{-5} M, b) X = Br, [MBA] = 1.1×10^{-4} M.

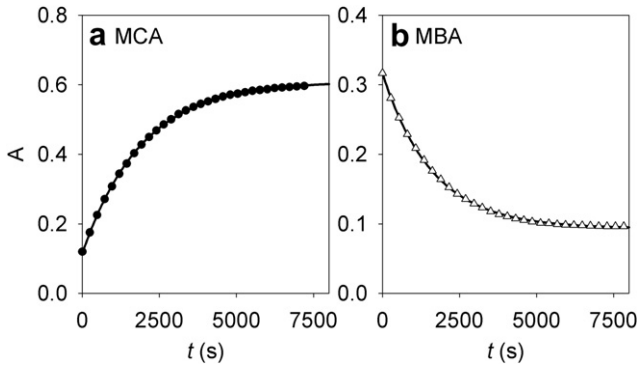


Fig. 2 – Kinetic profiles of the reaction of MXA with OH^-; T = 25.0 °C; a) X = Cl, $[OH^-] = 2.6 \times 10^{-2}$ M, $\lambda = 290$ nm; [MCA] = 3×10^{-5} M; b) X = Br, $[OH^-] = 6.5 \times 10^{-3}$ M, [MBA] = 4×10^{-5} M, $\lambda = 275$ nm.

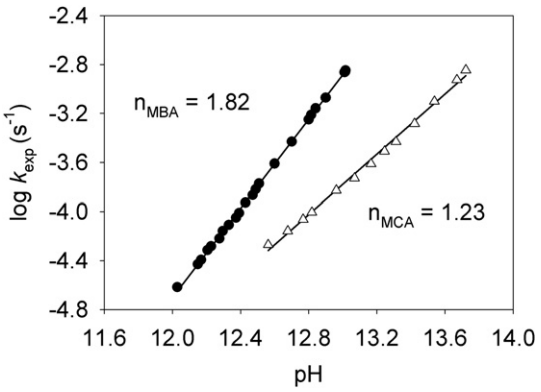


Fig. 3 – Variation in k_{exp} with pH. Reaction order with respect to [OH^-]. T = 25.0 °C.

MXA do not decompose to MOXA at the common pH levels of tap water, 6.5–8.5 being the values recommended by the US Environmental Protection Agency in the National Secondary Drinking Water Regulations (US-Cfr, 2002), or those of surface waters, since the global median pH value is 7.7, most average annual pH values are between 6.5 and 8.3, and very rarely exceed 9.0 (GEMStat, 2010). Therefore, the amount of time elapsed since water treatment is not expected to modify exposure to MXA or MOXA.

A situation worth noting could arise in the case of prechlorination followed by lime softening, where the pH values are raised to high levels such as those used in this work (pH > 10). At these high concentrations of OH^-, the formation of mucoxyhalic acids is kinetically significant. Whereas the environmental fate of MOXA is unknown, in the acidification following lime softening, they could undergo decarboxylation and evolve into malondialdehydes, which are known carcinogens.

The quadratic dependence of k_{exp} on [OH^-] suggests a mechanism involving two successive reactions with hydroxide ions. Such a dependence has often been documented in the hydrolysis of carbonyl compounds such as anilides (Biechler and Taft, 1957; Pollack and Bender, 1970), arylaminoacrolein (Ono et al., 1989), and acylpyrroles (Menger and Donohue, 1973) or sulfonyl compounds such as sultams

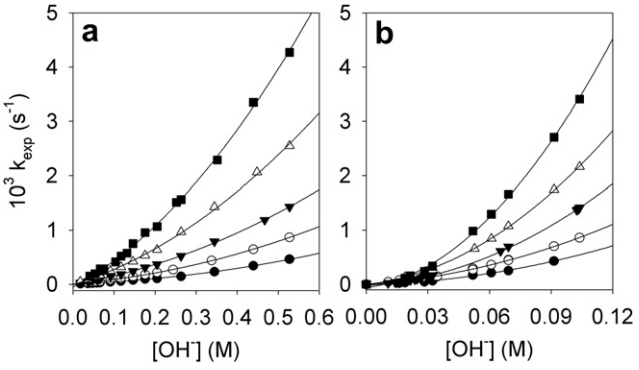


Fig. 4 – Fit of experimental values of k_{exp} to eq. (7). a) X = Cl; b) X = Br. ■ 35.0 °C, △ 30.0 °C, ▼ 25.0 °C, ○ 20.0 °C, ● 15.0 °C.

Table 1 – Rate Constants for the Reaction of MXA with OH^-.

T (°C)	MCA		MBA	
	$10^4 k_1^{OH}$ (M^{-1} s^{-1})	$10^4 k_2^{OH}$ (M^{-2} s^{-1})	$10^4 k_1^{OH}$ (M^{-1} s^{-1})	$10^2 k_2^{OH}$ (M^{-2} s^{-1})
15.0	3.5 ± 0.1	9.8 ± 0.5	7.0 ± 1.1	3.4 ± 0.3
20.0	7.0 ± 0.2	17.7 ± 0.4	13.4 ± 1.6	5.6 ± 0.2
25.0	13.0 ± 0.3	31.1 ± 0.7	20.8 ± 1.6	10.6 ± 0.2
30.0	22.9 ± 1.0	49.6 ± 2.3	35.0 ± 2.1	15.9 ± 0.3
35.0	41.2 ± 1.3	88.4 ± 3.2	59.6 ± 1.1	28.1 ± 1.2

(Baxter et al., 2000; King et al., 1996; Page, 2004). The existence of a second-order term is generally interpreted as coming from the reversible addition of the first hydroxide anion to the carbonyl group and the subsequent deprotonation of the hydroxyl group formed by the second OH^-, resulting in a dianion, which expels the leaving group.

The second-order term measured in this work is, to our knowledge, a novelty with respect to those described before, since in the reaction of mucohalic acids with hydroxide ions the leaving group is not located on the carbonylic carbon. Therefore, some variation in the accepted hydrolysis mechanism can be proposed, such as two successive attacks of the hydroxide ion on different electrophilic sites, the first one being a fast equilibrium and the second one the actual nucleophilic substitution. Alternatively, a deprotonation followed by substitution of the halogen atom could be proposed.

Since the only hydrogen atom in MXA is not expected to dissociate in the working pH interval, and, since aldehydes have high hydration constants, we suggest a possible reaction mechanism that could help to understand the observed results (Scheme 3).

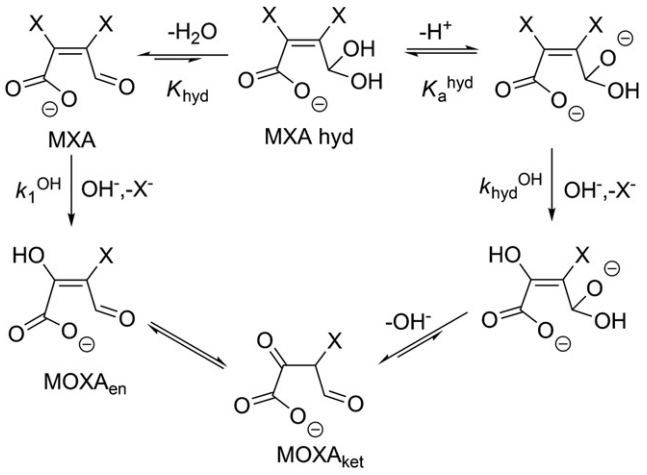
In the proposed mechanism, mucohalic acids react directly with hydroxide ions, with rate constant k_1^{OH} in a first-order reaction. At the same time, MXA can add water to form the corresponding hydrate, the equilibrium constant for the formation of the gem-diol being $K_{hyd} = MXA_{hyd}/MXA$. The hydrate can, in turn, undergo deprotonation (K_a^{hyd}); the sum of these two first steps is equivalent to the reversible attack of a hydroxide anion on the carbonyl group. The deprotonated hydrate can subsequently react with OH^- (k_{hyd}^{OH}). The predicted rate equation arising from this mechanism is eq. (8), which has both first-order and second-order terms, as observed experimentally.

$$\frac{d[MOXA]}{dt} = \left(k_1^{OH}[OH^-] + K_{hyd}K_a^{hyd}k_{hyd}^{OH}[OH^-]^2\right)[MXA] \quad (8)$$

The product $K_{hyd}K_a^{hyd}k_{hyd}^{OH}$ would equal the experimental rate constant k_2^{OH}. The large difference observed in k_2^{OH} for MCA

Table 2 – Activation Energies for k_1^{OH} and k_2^{OH}

	E_a (kJ mol^{-1})	
	k_1^{OH}	k_2^{OH}
MCA	90.0 ± 1.1	80.1 ± 1.5
MBA	77.3 ± 2.2	77.4 ± 2.4



Scheme 3 – Proposed reaction mechanism of MXA with OH^-.

and MBA can be explained in terms of a difference in k_{hyd}^{OH} (suggesting an inherently higher reactivity of MBA as compared with MCA), in K_a^{hyd} (suggesting that the hydrate of MBA is more acidic than that of MCA), or in K_{hyd} (suggesting greater electrophilicity of the aldehyde group in MBA) or any combination of the three.

To gain more information about the proposed mechanism, the hydration constants of mucohalic acids were determined using ^{1}H NMR spectroscopy.

3.2. *Hydration constants of mucohalic acids*

Since aldehydes in their hydrate form cannot react with nucleophilic sites in DNA, the hydration of aldehydes is also significant in their role as alkylating and potentially mutagenic and carcinogenic agents. For instance, at any given pH, 99.997% of chloral in aqueous solution is in its hydrate form, which significantly reduces its potential reactivity as an electrophile, and this in turn influence its genotoxic potential (Daniel et al., 1992; Haselkorn et al., 2006; Salmon et al., 1995; Seng et al., 2003).

The ^{1}H NMR spectra of mucohalic acids in neutral and basic aqueous solution show one major signal, belonging to the hydrogen atom of the aldehyde group (δ_{MCA} = 9.696 ppm; δ_{MBA} = 9.409 ppm). A minor signal is also observed, and its chemical shift (δ_{MCA} = 6.110 ppm; δ_{MBA} = 5.715 ppm), similar to that of gem-diols, hemiacetals, and acetals, suggests that this peak corresponds to the hydrate of the aldehyde group. The ratio of the two peak areas are time- and concentration- independent.

By introducing the peak areas in eq. (1), the values shown in Table 3 were obtained. The hydration constants are somewhat lower than those of other haloaldehydes, such as chloroacetaldehyde, trichloroacetaldehyde, 2-chlorobutyraldehyde or 2-bromobutyraldehyde, which have K_{hyd} values of $10^{1.6}$, $10^{4.45}$, $10^{1.2}$ and $10^{0.6}$, respectively (Bell, 1966; Gómez-Bombarelli et al., 2009; Hilal et al., 2005). This suggests that the presence of the double bond, together with the conjugation with the carboxylate group, decreases the electrophilicity of the aldehyde group, and hence the hydration constant. Nevertheless, the amount of hydrate in the equilibrium is significant, since close to 20% of MXA is present in this form. Because aldehyde hydrates lack the electrophilicity of the free aldehyde group, these values imply a decrease in the alkylating capacity of mucohalic acids.

Table 3 – Hydration Constants of Mucohalic Acids.

	K_{hyd} (unitless)
MCA	0.23 ± 0.04
MBA	0.17 ± 0.03

This high concentration of hydrate is also consistent with the hypothesis of hydrates playing a role in the proposed mechanism. Since the hydration constants of both compounds are very similar, the large difference in k_2^{OH} between MBA and MCA – one hundred-fold – could be due both to the acidity of the hydrates (K_a^{hyd}) and to the inherent reactivity of the deprotonated hydrate (k_{hyd}^{OH}). A higher k_{hyd}^{OH} for MBA should be expected since, as stated above, bromide is a better leaving group, and this tendency may be increased by the presence of one extra negative charge in the molecule, as in the deprotonated hydrate.

Whereas the experimental determination of K_a^{hyd} is challenging, standard procedures exist for the computational calculation of acid dissociation constants (Ho and Coote, 2010). We have estimated the values at the DFT B3LYP 6–31++G(d,p) level of theory with the Polarizable Continuum Model for solvation, using the Gaussian 03 suite of programs (Frisch et al., 2003). The pK_a^{hyd} values predicted were 23.0 for MCA and 23.6 for MBA. Absolute computational pK_a values are known to show significant errors systematic errors and, as regards to the present work, only the difference between MCA and MBA is of interest. Since both values are almost equal within the error of the methodology applied (~1 log unit), the large difference in k_2^{OH} can only be attributed to bromide as the better leaving group (k_{hyd}^{OH}).

4. Conclusions

From the present work, the following conclusions were drawn:

(i) At moderately basic pH, the reaction of mucohalic acids with OH^- to form mucoxyhalic acids is kinetically significant.

(ii) At the pH of natural or tap water (6.5 > pH > 8.5), mucohalic acids are not expected to degrade spontaneously. In cellular conditions (pH ~7.40), mucohalic acids do not yield mucoxyhalic acids. Thus, MOXA are not expected to participate in the *in vivo* genotoxicity mechanism of the acids.

(iii) The nucleophilic attack of hydroxide ions on mucohalic acids occurs through a combination of two paths, one of them first-order in [OH^-] and the other second-order in [OH^-], and could occur through the deprotonation of the hydrate of mucohalic acids.

(iv) The hydration constants of mucohalic acids –0.23 and 0.17 for mucochloric and mucobromic acid respectively – correspond to very significant hydrate concentrations. Since aldehyde hydrates are not electrophilic, these values imply a decrease in the alkylating capacity of mucohalic acids.

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