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RESEARCH NOTE

FACTORS ON THE FORMATION OF STRONG MUTAGEN [3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE] MX BY CHLORINATION OF SYRINGALDEHYDE

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Abstract—Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde) was chlorinated in the laboratory at different reaction time, temperature, pH and chlorine dose, and MX was determined and quantified by GC/MS. The finding suggests that the optimal chlorination conditions are as follows: temperature = 298 K, pH = 5, Cl:syrin. (mol:mol) = 9:1. We also found that the formation of MX increased with the syringaldehyde concentration in a linear relationship. In addition, when syringaldehyde was chlorinated with a different chlorine dose in an alkaline condition, no MX was detected. This study will conduce to elucidating the kinetic process for the formation of MX from syringaldehyde and will be of great help for better control of MX in drinking water. © 2000 Elsevier Science Ltd. All rights reserved

Key words—mutagen, MX, drinking water, precursor, syringaldehyde, chlorination

INTRODUCTION

MX, a by-product of the chemical reactions that occur in chlorinated drinking water, was found to be one of the most potent direct-acting mutagens ever tested in Ames tests strain TA 100 (Hemming *et al.*, 1986), and accounted for up to 67% of the overall mutagenity in chlorine disinfected drinking waters (Smeds *et al.*, 1997). MX was also found to be a mutagen in bacteria (Holmbom *et al.*, 1984) and mammalian cells (Jansson and Hyttinen, 1994), to be a potent carcinogen and cause tumors at doses in both male and female rats (Komulainen *et al.*, 1997), and to induce DNA damage in HL-60 cells (Marsteinstredet *et al.*, 1997). It is generally assumed that MX is a product of the reaction of chlorine with humic substances (Backlund *et al.*, 1988, 1989; Kronberg *et al.*, 1988; Charles *et al.*, 1992; Långvik and Hormi, 1994), phenolic compounds (Långvik *et al.*, 1991a), and some amino acids (Horth *et al.*, 1990). Xu *et al.* (1997a,b) fractionated organic matter from Taihu Lake by sorption on a series of resin absorbents according to the method of Leenheer (1981) with some modifications, compared the yield of MX of each fraction by GC/MS, and characterized humic substances by analyzing their oxidation products. From that study (Huixian *et al.*, 1999), we presumed and selected probable precursors, and found the yield of MX produced by syringaldehyde per mole was highest. MX undergoes several reactions and transformations in water. The aqueous stability of the compound has been studied by Backlund *et al.* (1989). The reactions/transitions, which are highly pH dependent, include tautomeric transformations from ring to dissociated open chain form, isomerization of MX to its E-form and degradation of MX. In this work, we have further studied the influence of chlorination (reaction time, temperature, pH and chlorine dose) on the formation of MX by GC/MS in a solution containing 15 mg syringaldehyde and the relationship between the yield of MX and syringaldehyde concentration. It is conducive to screening the precursor of MX to a larger extent and elucidating the kinetic process of the formation of

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MX. And the results may be also helpful for the better control of MX formation in drinking water.

MATERIALS AND METHODS

Chlorination of syringaldehyde

An 0.2 l buffer solution containing 15 mg of syringaldehyde was chlorinated by the addition of freshly prepared sodium hypochlorite/hypochlorous acid solution in glass bottle. Chlorination was carried out in the dark. The following ranges of conditions were studied: pH, 2–9.5(pH = 2–4: CH_3COONa and HCl; pH = 5: CH_3COONa and CH_3COOH; pH = 6–9.5: H_3PO_4, NaH_2PO_4 and Na_2HPO_4); temperature, 278–303 K; reaction time, 2–24 h; syringaldehyde, 3–15 mg; Cl:syrin. (mol:mol) ratio, 3.0–19–18.0.

Analysis of syringaldehyde

After chlorination, the aqueous solution was quenched with a certain quantity of $NaAsO_2$ and was extracted three times with 20, 10, 10 ml of dichloromethane. The organic phase was concentrated to a small volume by rotary vacuum evaporator. Quantitative analysis was performed with gas chromatography-mass spectrometry by the internal standard method in selective ion monitoring. Identification of syringaldehyde was based on position matching of retention time and some characteristic ions, m/z 96, 111, 139, 167, 182 ions.

Analysis of MX

After chlorination, the aqueous solution was quenched with a certain quantity of $NaAsO_2$ and was extracted three times with 20, 10, 10 ml of newly distilled diethyl ether at pH = 2. The organic phase was concentrated to a small volume by rotary vacuum evaporator and evaporated to dryness by a gentle stream of nitrogen gas. The extracts were methylated by 0.6 ml saturated BF_3 in methanol in a 95°C water bath for 1 h. The reaction mixture was neutralized with 1 ml 2% (w/v) $NaHCO_3$, and then extracted three times with 0.5, 0.4, 0.3 ml n-hexane sequentially. The n-hexane extract was concentrated to 0.1 ml under a gentle nitrogen gas flow for later analysis. Quantitative analysis was performed with gas chromatography-mass spectrometry by the internal standard method in selective ion monitoring. The gas chromatography column was 30 m × 0.25 mm × 0.25 μm HP-5 fused silica capillary column. Identification of methylated MX was based on position matching of retention time and some characteristic ions, m/z 147, 107, 199, 201 and 203 ions (Huixian *et al.*, 1995).

RESULTS AND DISCUSSION

Disappearance of syringaldehyde after chlorination

At the following ranges of conditions: pH, 2–9.5; temperature, 278–303 K; reaction time, 2 h; syringaldehyde, 15 mg; Cl:syrin. (mol:mol) ratio, 3.0–18.0, more than 99% syringaldehyde disappeared. At the chlorination condition (Cl:syrin.) (mol:mol) ratio, 6.0; time, 5 min; pH, 5; temperature, 298 K), syringaldehyde disappeared nearly to 99%. It means that syringaldehyde reacts with chlorine very easily.

Influence of reaction time

Changes of yields of MX with time are shown in Figs 1 and 2. Figure 1 shows that MX increases with the reaction time at Cl:syrin. (mol:mol) ratio of 6.0 in six different temperatures (278–303). As syringaldehyde reacted with chlorine in a short time, there is no direct relationship between the disappearance of syringaldehyde and the formation of MX. In Fig. 2, MX decreases with the reaction time at Cl:syrin. (mol:mol) ratio of 9.0–18.0, which suggested that MX is formatted rapidly and degraded with excess chlorine at higher Cl:syrin. ratio (Schenck *et al.*, 1990).

Influence of chlorine dose

The effect of the applied chlorine dose on the formation of MX was investigated using a wide range of chlorine to syringaldehyde mole ratio from 3:1 to 18:1 (Fig. 3). The formation of MX is found to

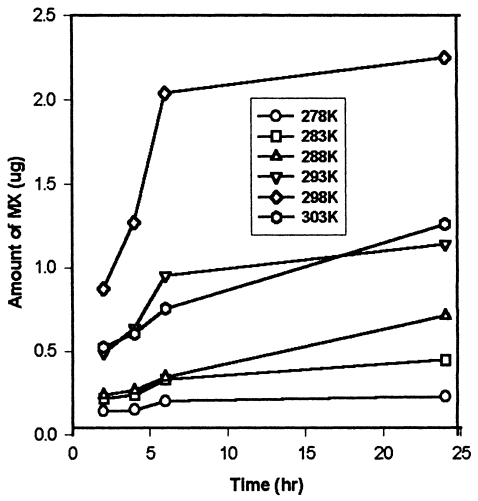

Fig. 1. Formation of MX as a function of reaction time. (pH = 5, Cl:syrin. = 6:1).

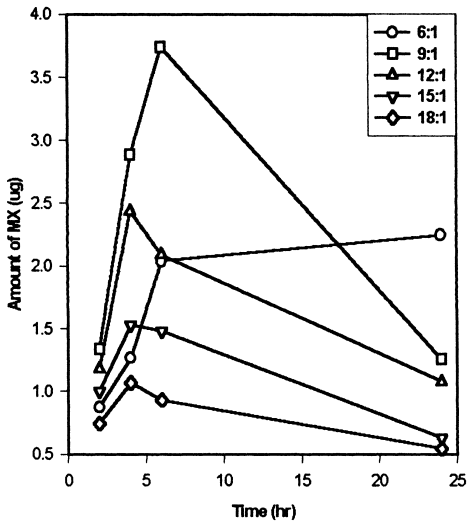

Fig. 2. Formation of MX as a function of reaction time. (pH = 5, T = 298 K).

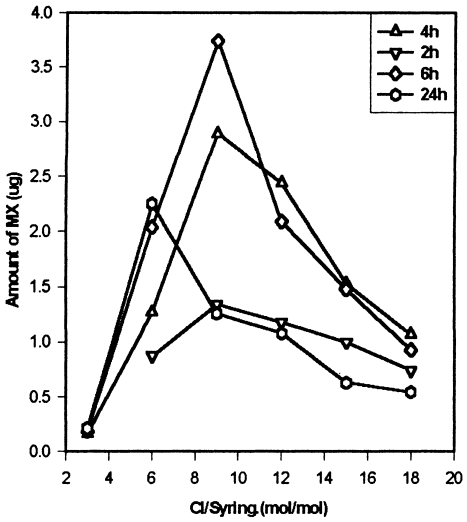

Fig. 3. Formation of MX as a function of chlorine dose (pH = 5, T = 298 K).

depend strongly on the applied chlorine concentration: it increased as the mole ratio was increased to 9:1, and decreased as the weight ratio was increased to 18:1. This may indicate that excess chlorine reacts further with formed MX and produces other compounds (Schenck *et al.*, 1990).

Influence of pH

The effect of pH on MX formation is very complex due to the complicated reaction mechanism (Fig. 4). At acidic conditions, we can detect the compound of MX. And the optimal pH value for the formation of MX is 5.0, which is contradictory to the results reported by Långvik *et al.* (1991a). It may be because that when the pH value decreases, MX produced from syringaldehyde reacts with chlorine and produces additional compounds. The formation of MX is usually favored by chlorination at acidic conditions, but as to easy degrading organics such as syringaldehyde, the MX yield decreased at a lower pH condition. And at alkaline and neutral conditions, we cannot detect the MX. In this case, the syringaldehyde reacts with chlorine and produces other compounds than MX. And at pH = 7.2, the formed MX reacts quickly with chlorine (Långvik *et al.*, 1991b). Further study in this area is needed.

Influence of temperature

The range of drinking water temperature is usual according to the real chlorination temperature of our drinking water. In this study, we used six different temperatures (278, 283, 288, 293, 298 and 303 K) to investigate the formation of MX. The yield of MX against T was plotted in Fig. 5. At lower temperatures (278 or 283 K), the formation of MX is small. As the reaction temperature increases, the formation of MX becomes more rapid and reaches the largest quantity at 298 K, but when the temperature increases continually, the formation of MX becomes less. It may be because that at high temperatures the procedure of the formation of MX by the chlorination of syringaldehyde becomes more complicated and comprises more unknown reactions.

Influence of syringaldehyde concentration

Five 0.2 l buffer solution (pH = 5.0) containing different amount of syringaldehyde (3, 6, 7.5, 9 and 12 mg) was chlorinated at a chlorine:syringaldehyde ratio of 9:1 and 298 K. Two contact times (2 and 4 h) are investigated. Figure 6 shows the linear relationship between MX and the concentration of

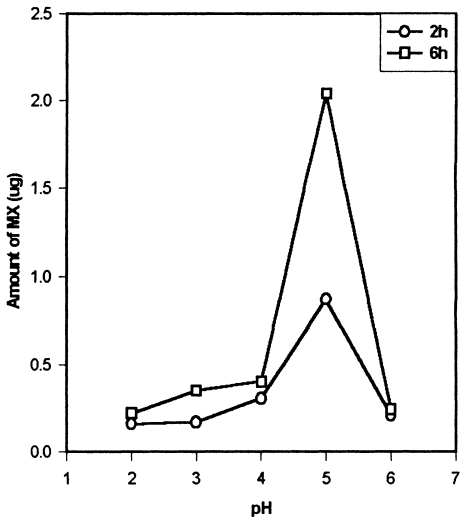

Fig. 4. Formation of MX as a function of pH (T = 298 K, Cl:syrin. = 9:1).

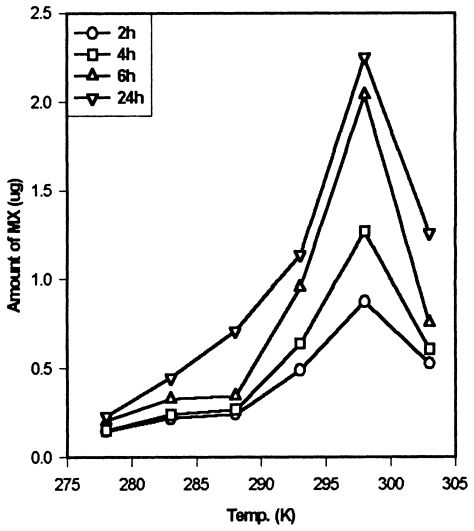

Fig. 5. Formation of MX as a function of temperature. (pH = 5, Cl:syrin. = 6:1).

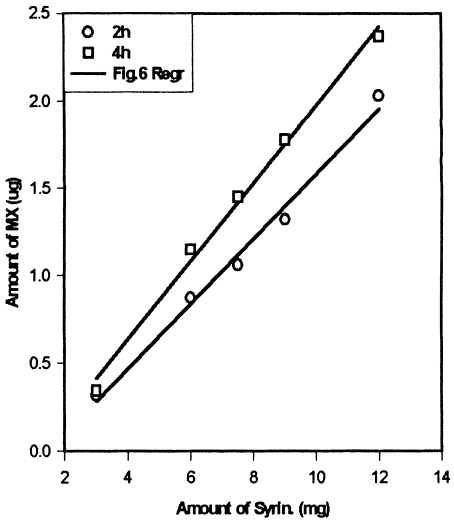

Fig. 6. Formation of MX as a function of syrin. concentration (pH = 5, T = 298 K, Cl:syrin. = 9:1).

syringaldehyde. It indicates that the formation of MX per weight of syringaldehyde is constant and does not change with the concentration of syringaldehyde in the other conditions.

CONCLUSION

According to our previous research work (Xu *et al.*, 1997a,b; Huixian *et al.*, 1999), syringaldehyde is the major producer of oxidized humic materials and on the basis of our result of screening the precursors of MX, syringaldehyde is the compound which is chlorinated to yield the maximum MX at the same chlorination conditions. From our study, the relationship between MX and the concentration of syringaldehyde is linear and the optimal conditions for the formation of MX by chlorination of syringaldehyde are as follows: pH, 5.0; Cl:syrin. (mol/mol), 9.1: T, 298 K and t, 6 h. Syringaldehyde reacts with chlorine very quickly and there is no direct relationship between the disappearance of syringaldehyde and the formation of MX. The most efficient way to decrease the MX of drinking water is to make our endeavor to wipe off the humic materials before chlorination. By altering the chlorination conditions, we can also reduce the formation of MX. The results of this study will also be helpful for further studying the kinetic process of the formation of MX and be conducive to screening the precursors of MX to a larger extent.

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