Formation of iodinated trihalomethanes during chlorination of amino acid in waters

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Abstract

Chlorination is essential to provide safe drinking water. However, this process leads to the formation of disinfection byproducts (DBPs). In this study, tryptophan (Trp) has been selected as a precursor to conduct the chlorine disinfection. Moreover, the factors that affect the formation of trihalomethanes (THMs) and iodinated trihalomethanes (I-THMs) are investigated. The formation pathway of Trp chlorination is proposed based on the intermediate products identified.

According to the experimental results, the formation of THMs and I-THMs during Trp chlorination fitted a new first-order kinetic model. The dosage of chlorine, temperature, pH and the ratio of bromide and iodide had major influence on the formation of THMs and I-THMs during chlorination. In addition, the inhibition of luminescent bacteria \textit{Vibrio fischeri} in the water sample increased during Trp chlorination.

Keywords: amino acid, chlorination, trihalomethanes, iodinated trihalomethanes, tryptophan

1. Introduction

Currently, provision of safe drinking water has been one of the most concerns for human beings and the most basic guarantee of the life quality (Plewa and Wagner, 2015). Biological safety of drinking water has been always ensured by disinfection;
moreover, the chlorine as a disinfectant is the most widely used disinfection technology because it is effective, economic and provides distribution residual within the distribution system (He et al., 2016; Dong et al., 2017; How et al., 2017; Jiang et al., 2017; Zhang et al., 2018). Although chlorine is effective in deactivating pathogens, it leads to concern regarding the formation of disinfection byproducts (DBPs) (McMahan et al., 2016; Du et al., 2017). Since the discovery of DBPs in chlorinated drinking water in the early 1970s, many researchers have found the source of precursors, understood the formation of DBPs, and controlled them (Richardson et al., 2007; Postigo and Richardson, 2014; Chu et al., 2016b; How et al., 2017). During the chlorination, chlorine reacts with precursors, mainly dissolved organic matter, to form various DBPs (Han, Jiarui et al., 2017). Thus far more than 600 DBPs have been identified (Richardson et al., 2007).

Amino acids, which can be found in all types of natural water, are significant portions of hydrophilic natural organic matter (NOM) in drinking water sources (Shan et al., 2012; Cermakova et al., 2017). The concentration of amino acids can range from 20 to 10,000 μg/L, accounting for 2~13% of dissolved organic carbon and up to 75% of dissolved nitrogen in natural waters (Thurman, 1985; Hureiki et al., 1994; Westerhoff and Mash, 2002; Hong et al., 2009). Amino acids are nontoxic compounds acting mainly as blocks of microorganisms’ DNA, RNA, proteins and so on, but their occurrence in the treated water is undesirable, which causes a large number of problems across water treatment process (Hureiki et al., 1994; Froese et al., 1999; Gagnon et al., 2000; Freuze et al., 2005). Amino acids have been highlighted as key precursors in the formation of a variety of DBPs, including trihalomethanes (THMs), haloacetaldehydes, haloacetonitriles, haloacetamides and halonitromethanes (Scully and White, 1991; Bond et al., 2012; Shah and Mitch, 2012; Yao et al., 2017). Amino
acids as identified precursors of DBPs not only contribute to the formation of DBPs but also have difficulty in being removed during the conventional treatment processes (Dotson and Westerhoff, 2009; Yao et al., 2017). Tryptophan (Trp) is a kind of natural amino acid present in water containing organic-N compounds such as proteins, peptides, foods, humics and algae (Szajdak and Österberg, 1996). Trp, asparagine and aspartic acid are known to produce significant amounts of dichloroacetonitrile during chlorination (Bond et al., 2009), which may have adverse effects on human health. Moreover, Trp, containing an indole function group, has higher chlorine demand and THM formation potential (Wang et al. 2007).

Generally, DBPs include the most abundant group THMs produced during chlorination (Richardson, 2003). The common THMs consist of chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform (TBM). TCM, the most commonly identified THM, was the first carbon-based DBP (C-DBP) group to be detected in chlorinated drinking water in 1974 (Bellar et al., 1974), and has been researched extensively because of its potential carcinogenicity, high detection rate, and high concentration (Richardson et al., 2007). According to the discovery of their cytotoxicity and genotoxicity, the US Environmental Protection Agency (USEPA) has established a maximum contaminant level (MCL) of 80 µg L\(^{-1}\) for four THMs (Usepa, 2006; Ye et al., 2013). The sanitary standard for drinking water in China (GB 5749-2006) has specified the MCL of 0.06 mg L\(^{-1}\), 0.06 mg L\(^{-1}\), 0.1 mg L\(^{-1}\) and 0.1 mg L\(^{-1}\) for TCM, BDCM, DBCM and TBM, respectively.

However, when iodide (I\(^{-}\)) (from natural sources, seawater intrusion or brines) exists, I\(^{-}\) is oxidized to hypoiodous acid (HOI) during treatment, and then HOI can either react with NOM, which can lead to the formation of iodinated disinfection byproducts (I-DBPs), or be oxidized to IO\(_3^−\) (Bichsel and Gunten, 2000b; Plewa et al.,
2004; Richardson et al., 2008; Jones et al., 2012a; Gong and Zhang, 2013; Ye et al., 2013). Iodinated trihalomethanes (I-THMs), including dichloroiodomethane (CHClI₂), diiodochloromethane (CHClI₂), bromochloroiodomethane (CHBrClI), bromodiiodomethane (CHBrI₂), dibromodiiodomethane (CHBr₂I) and iodoform (CHI₃), were identified in drinking water as early as 1975 (Hansson et al., 1987). The I-THMs caused medicinal or pharmaceutical taste and odor problems in drinking water (Cancho et al., 2000). Apart from I-THMs, other I-DBPs have also been detected in drinking water in recent years, including iodinated haloacetamides (I-HAcAms) (Chu et al., 2012), iodinated haloacetic acids (I-HAAs) (Krasner et al., 2006) and polar I-DBPs (Ding and Zhang, 2009). Recent studies have shown that I-DBPs are more cytotoxic and genotoxic than brominated and chlorinated DBPs (Plewa et al., 2004; Jones et al., 2012b; Tian et al., 2014; Pan et al., 2016; Xia et al., 2017). I-DBPs have been reported to occur in drinking water at µg L⁻¹ level, and the enhanced cytotoxicity and genotoxicity in mammalian cells are affirmed theoretically compared to their brominated and chlorinated analogues (Richardson et al., 2008).

At present, there are an increasing number of researches studied on six I-THMs. I-THMs, especially CHI₃, which constitute the majority of I-DBPs, can result in medicinal tastes and odors in drinking water due to their low threshold concentrations (Bichsel and Gunten, 2000a; Cancho et al., 2001; Zhang et al., 2015). THMs are the most regulated DBPs; however, there are very few researchers studying on four regulated THMs and six I-THMs during Trp chlorination. Therefore, the amino acid Trp was selected as a precursor to conduct the chlorine disinfection. Moreover, the analysis of the four regulated THMs and six I-THMs was conducted on a gas chromatograph with an electron capture detector (GC-ECD).

The main objectives of the study were as follows: (1) to investigate the DBPs of
Trp chlorination, and study the formation of four regulated THMs and six I-THMs; (2) to examine the effect of impact factors (the dosage of chlorine, temperature, pH and the ratio of bromide ($\text{Br}^-$) to iodide ($\text{I}^-$)) on the formation of THMs and I-THMs; and (3) to propose the pathway of Trp chlorination based on the identified DBPs.

2. Materials and methods

2.1 Chemicals

All chemicals were at least of analytical grade except as noted. Five I-THM standards, including $\text{CHCl}_2\text{I}$ ($\geq 95\%$), $\text{CHClI}_2$ (90~95\%), $\text{CHBrClI}$ ($\geq 95\%$), $\text{CHBrI}_2$ (90~95\%) and $\text{CHBr}_2\text{I}$ (90~95\%), were obtained from Cansyn Chemical Crop (Canada). A mixture of four THMs (GC grade) was obtained from Supelco (Supelco Park, PA, USA). $\text{CHI}_3$ (99\%), Trp, sodium iodide (NaI, $\geq 95\%$), methyl tert-butyl ether (MTBE), methanol, acetonitrile, n-hexane and phosphoric acid were purchased from Sigma-Aldrich (USA). Sodium hypochlorite and ascorbic acid ($\geq 99\%$) were purchased from Aladdin (China). Sodium bromide, sodium thiosulfate, sodium dihydrogen phosphate, disodium phosphate, sodium hydroxide and ammonium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) without further purification. All solutions were prepared by ultrapure water (NW Ultrapure water system, Heal-Force), which was obtained from a Milli-Q system with resistivity $> 18 \text{ M}\Omega\cdot\text{cm}$.

2.2 Analytical methods

The detection of bromide and iodide ions was tested using ICS-2000 (Dionex, USA), and the analysis conditions are shown in Table S1. The detection of free residual chlorine was tested using DR2800 (HACH). The detection of THMs was simplified by using Purge and Trap (P&T, Atomx Automated VOC Sample preparation system, TELEDYNE TEKMAR, USA) as a pretreatment technology. The
operating parameters were as follows: temperature at 30 °C, purge time at 15 min, 
 purge flow at 40 mL min$^{-1}$, dry purge time at 1.5 min, dry purge flow at 100 mL min$^{-1}$, 
desorb temperature at 210 °C, desorb time at 3 min, heat temperature at 250 °C, heat 
time at 5 min and sample volume at 30 mL. The detection of I-THMs was simplified 
by using liquid–liquid extraction (LLE) as a pretreatment technology. A 10 mL aliquot 
of water sample was extracted by addition of 2 mL of MTBE. The sample was shaken 
in a mechanical shaker for 5 min and then was allowed to stand for 5 min.

The separation and identification of THMs and I-THMs were detected by 
GC-ECD (GC-450, VARIAN), equipped with a splitless injector and a DB-5 capillary 
column (30 m × 0.25 mm × 0.25 µm, Agilent). The operating parameters of THMs were as follows: The oven was held at 60 °C for 1 min, then was ramped at 20 °C 
min$^{-1}$ to 250 °C; the injector and detector temperatures were 150 °C and 300 °C, 
respectively; nitrogen was used as carrier gas with a flow rate of 1 mL min$^{-1}$.

Moreover, the operating parameters of I-THMs were as follows: The oven was held at 
40 °C for 10 min, then was ramped at 15 °C min$^{-1}$ to 260 °C; the injector and detector 
temperatures were 200 °C and 290 °C; nitrogen was with a flow rate of 1 mL min$^{-1}$.

The limit of detection (LOD) for THMs and I-THMs detected by GC-ECD was 
shown in Table S2.

The intermediate products by Trp chlorination were detected by gas 
chromatography mass spectrometer (GC-MS-SCAN, GC2010 Plus/MS-OP2010 SE), 
equipped with a splitless injector and a Rtx-5 MS column (30 m × 0.25 mm × 0.25 
µm, Agilent). The operating parameters were as follows: The oven was held at 35 °C 
for 3 min, then was ramped at 3 °C min$^{-1}$ to 50 °C and then was ramped at 5 °C min$^{-1}$ 
to 260 °C; the injector, detector and ion source temperatures were 260 °C, 250 °C and 
280 °C, respectively; nitrogen was used as carrier gas with a flow rate of 1 mL min$^{-1}$. 
2.3 Experimental procedures

In order to investigate the byproducts of Trp chlorination and study the formation of THMs and I-THMs, the experiments were conducted as follows: 2 mg L\(^{-1}\) as Cl\(_2\), reaction temperature of 25 °C, pH of 7, both the concentrations of Br\(^-\) and I\(^-\) at of 200 µg L\(^{-1}\) and the initial concentration of Trp of 1 mg L\(^{-1}\). The reaction was conducted in a 1 L brown glass bottle, and ultrapure water was used as the reaction substrate.

First, 1 L of phosphate buffer solution (pH = 7) was placed in brown glass bottle and 8 mL of solution was removed to waste bucket by pipette. Then, 1 mL of Trp solution, bromide solution and iodide solution were, respectively, added into the container. Second, the mixture was stirred by magnetic heating stirrer (1000 r min\(^{-1}\)) with an electronic intelligence control instrument to maintain the reaction temperature at 25 °C. After thorough mixing, 5 mL of sodium hypochlorite solution was added into the container. The mixture was still stirred by magnetic heating stirrer. Samples were taken at 0, 0.5, 1, 2, 4, 6, 12, 24 and 48 h. For sampling time, the experimental steps were as follows: (1) 10 mL of sample was taken immediately for residual chlorine determination; (2) when 30 mL of sample was collected, excess sodium thiosulfate was added as a quenching agent to stop any further reactions with chlorine for THM determination; (3) when 10 mL of sample was collected, excessive sodium thiosulfate was added as a quenching agent to stop any further reactions with chlorine for I-THM determination.

The experiments for the analysis of intermediates were conducted by Trp chlorination. After running for certain reaction time (0, 1, 2, 4, 6, 12 and 24 h), 1 L samples were collected by pretreatment procedure for GC-MS-SCAN (GC2010 Plus/MS-OP2010 SE) analysis. When the samples were collected, excess sodium
thiosulfate was added as a quenching agent to stop any further reactions with chlorine.

The toxicity of the samples was tested by using a Microtox Model 500 Analyzer
(Strategic Diagnostic Inc. US.). Water sample of 2.5 mL was taken in a 10 mL brown
glass bottle at different time points. According to the ASTM standard method, the
toxicity was assessed by the Micro Tox “Acute” toxicity test with the luminescent
bacteria Vibrio fischeri (freeze dried) (Ye et al., 2011; Barhoumi et al., 2016).

3 Results and discussion

3.1 Investigation of the background levels of THMs and I-THMs in real waters

Water samples were collected from five sampling sites in Zhejiang Province,
China. Sampling sites chosen were the main sources for drinking water. Sample 1 and
sample 2 were from reservoirs, sample 3 and sample 4 were from drinking water
plants and sample 5 was from river. Before chlorination, 1L water samples were
filtered through a 0.45 µm filter. After the solid particles filtered in the water sample,
pH, ultraviolet-254 (UV254), dissolved organic carbon (DOC) and the concentration
of Br⁻ and I⁻ were measured. The basic water quality index of five samples is
shown in Table S3.

Cl₂ (2 mg L⁻¹) was added to the water sample and the reaction temperature was
25 °C. All five samples produced both THMs and I-THMs by chlorination. Fig. 1(a)
shows the formation of THMs of five samples chlorination after 48 h and Fig. 1(b)
shows the formation of I-THMs of five samples chlorination after 48 h. The yields of
total THMs and total I-THMs in sample 1 were 17.69 µg L⁻¹ and 5.36 µg L⁻¹,
respectively; the yields of total THMs and total I-THMs in sample 2 were 36.11 µg
L⁻¹ and 10.98 µg L⁻¹, respectively; the yields of total THMs and total I-THMs in
sample 3 were 25.20 µg L⁻¹ and 10.63 µg L⁻¹, respectively; the yields of total THMs
and total I-THMs in sample 4 were 29.52 µg L⁻¹ and 10.71 µg L⁻¹, respectively; the
yields of total THMs and total I-THMs in sample 5 were 19.97 µg L\(^{-1}\) and 9.60 µg L\(^{-1}\), respectively.

It was seen from Table S3 that the DOC value of samples 2 and 4 was higher than that of other samples. Correspondingly, the yields of THMs of sample 2 and 4 by chlorination were more than that of other samples, which indicated that the formation of THMs was greatly dependent on the DOC value of water. High DOC value indicates that there are more dissolved organic DBP precursors in source water, which contributes to forming more THMs. Besides, the concentration of Br\(^{-}\) in sample 2 was significantly lower than other samples and the production of TBM was the least, which showed that the concentration of Br\(^{-}\) in water influenced the formation of Br-THMs. The concentration of I\(^{-}\) in five samples was all below 10 µg L\(^{-1}\); the type and proportions of I-THMs produced by each samples were different, mainly producing CHI\(_3\), CHCl\(_2\)I, CHClI\(_2\) and CHBrI\(_2\). CHCl\(_2\)I accounted for 83.16%, 91.12%, 89.74%, 46.71% and 56.74% of the total I-THMs in five samples, respectively. The amount of CHCl\(_2\)I was the most in the total I-THMs. Moreover, the yields of CHCl\(_2\)I produced by water sample 2 and water sample 3 were significantly larger than other water samples. While the yields of CHI\(_3\) produced by water sample 4 and sample 5 were larger than other water samples. It was mainly because each water sample contained different types and contents of organics. The results of the present study show the typical levels of THMs and I-THMs in surface water samples.

**Fig. 1.** The formation of (a) THMs and (b) I-THMs by chlorination of five samples \([\text{[free chlorine]} = 2 \text{ mg L}^{-1}; \ T = 25 ^\circ \text{C}]\)

3.2 The chlorination of Trp

3.2.1 The formation of THMs and I-THMs

**Fig. 2** shows the formation of THMs and I-THMs during Trp chlorination. THMs
and I-THMs formed during Trp chlorination increased with the increase in time. The reaction was stable after 24 h and the production of total THMs and total I-THMs at 48 h was 20.94 µg L\(^{-1}\) and 27.21 µg L\(^{-1}\), respectively. The production of I-THMs was 1.3 times of the production of THMs.

New first-order reaction kinetic equations are shown in Eq. (1) and Eq. (2):

\[
\ln(C_e - C_t) = \ln C_e - k \cdot t \quad (1)
\]

\[
C_t = C_e \left(1 - e^{-kt}\right) \quad (2)
\]

where \(C_e\) is the final concentration of reaction, \(C_t\) is the reactant concentration at time \(t\), \(k\) is the reaction rate constant and \(t\) is the reaction time.

The generated curves of THMs and I-THMs are fitted by Eq. (2), and the fitting curves are shown in Fig. 2. The linear regression coefficient \(R^2\) of the two fitting curves was greater than 0.98 for both THM and I-THM, which indicated that the their reactions by Trp chlorination fitted a new first-order reaction kinetic model. The kinetic equations were \(\frac{d\text{[THMs]}}{dt} = 0.246 \cdot \text{[THMs]}\) and \(\frac{d\text{[I-THMs]}}{dt} = 0.172 \cdot \text{[I-THMs]}\), and the formation rates of THMs and I-THMs were 0.246 h\(^{-1}\) and 0.172 h\(^{-1}\), respectively. It was found that the formation rate of THMs was faster than that of I-THMs, but the production of THMs was lower than that of I-THMs, which showed the formation mechanisms of THMs and I-THMs were obviously different. The production of I-THMs was formed because of the presence of \(\Gamma\) in water. HOCl was more oxidative than HOI (Bichsel and Gunten, 2000a; Richardson et al., 2008), so \(\Gamma\) was rapidly oxidized to HOI by HOCl and HOI was more likely to react with amino acids to form I-THMs.

**Fig. 2.** The generated curves of (a) THMs and (b) I-THMs by Trp chlorination ([free chlorine] = 2 mg L\(^{-1}\); \(T = 25^\circ\text{C}\); \(\text{pH} = 7\); \([\text{Br}^-] = 200 \mu\text{g L}^{-1}\); \([\Gamma] = 200 \mu\text{g L}^{-1}\); \([\text{Trp}] = \))
3.2.2 Effect of the dosage of chlorine on the formation of THMs and I-THMs

In the reaction process, the formation of THMs and I-THMs was stable at 24 h. Therefore, the sampling end point was selected at 24 h in the following experiments.

Fig. 3(a) and Fig. 3(b) show the formation of THMs and I-THMs in different dosage of chlorine during Trp chlorination. As shown in Fig. 3(a), the formation of THMs increased with the increase in chlorine. When the dosage of chlorine increased from 0.7 mg L$^{-1}$ to 4 mg L$^{-1}$, the concentration of THMs increased from 4.97 µg L$^{-1}$ to 32.01 µg L$^{-1}$. The proportion of TCM was continuously increasing, which was from the first 28% to 37%. With the increase in chlorine, it is more easily for heterocyclic structure of Trp to occur ring-opening reaction and substitution reaction and to promote the formation of TCM (Hong et al., 2009). Thus, the increase of chlorine played a significant role in improving the formation of THMs.

However, Fig. 3(b) shows that the formation of I-THMs exhibited an increasing phase then decreasing phase with the increase in chlorine. When the dosage of chlorine was 2 mg L$^{-1}$, the concentration of I-THMs was 27.17 µg L$^{-1}$, which reached the maximum. In addition, the major species of formed I-THMs was CHI$_3$, which accounted for 42.14% in sample chlorinated with 2 mg/L as Cl$_2$. While the dosage of chlorine was 4 mg L$^{-1}$, the formation of I-THMs significantly reduced to 8.54 µg L$^{-1}$.

It is well-known that I$^-$ can be oxidized to HOI by HOCl (Bichsel and Gunten, 2000b; Plewa et al., 2004; Richardson et al., 2008). Previous research has shown that 89% of I$^-$ would be oxidized to iodate by chlorine (Bichsel and Gunten, 2000b). However, as the dosage of chlorine was low, most of I$^-$ were oxidized to HOI and HOI could exist longer to react with NOM during chlorination, which contributed to higher amount of I-THM formation, especially CHI$_3$. When chlorine dosage increased
over a certain dose, more and more $\Gamma$ would be oxidized to iodate, which led to a significant reduction of I-THM formation in the decreasing order of $\text{CHI}_3 > \text{CHClI}_2 > \text{CHBrI}_2 > \text{CHBr}_2I > \text{CHBrClI}$.

**Fig. 3.** Effect of the dosage of chlorine on (a) the formation of THMs and (b) the formation of I-THMs ($T = 25 \, ^\circ\text{C}; \text{pH} = 7; [\text{Br}^-] = 200 \, \mu\text{g L}^{-1}; [\Gamma] = 200 \, \mu\text{g L}^{-1}; [\text{Trp}] = 1 \, \text{mg L}^{-1}$)

### 3.2.3 Effect of temperature on THMs and I-THMs formation

As shown in Fig. 4(a) and Fig. 4(b), with the increase in temperature from 15 °C to 35 °C, the formation of THMs and I-THMs also increased and the generation tendency was basically the same. The formation of THMs increased from 10.45 µg L$^{-1}$ to 28.7 µg L$^{-1}$ and the formation of TCM significantly increased from 3.32 µg L$^{-1}$ to 10.38 µg L$^{-1}$. As for the formation of I-THMs, it increased from 15.8 µg L$^{-1}$ to 34.17 µg L$^{-1}$. Among the total I-THMs, $\text{CHI}_3$ produced most. The proportions of $\text{CHI}_3$ were 48.2%, 43.5%, 49.8%, 51.8% and 52.5% at temperature of 15 °C, 20 °C, 25 °C, 30 °C and 35 °C, respectively. For endothermic reactions, the increase in temperature contributes to increasing the reaction kinetic energy and promoting the activation of molecules. Therefore, the increase in temperature would promote the reaction and increase the formation of THMs and I-THMs. Recent study shows that the increase in temperature promotes bromination, chlorination and iodination (Chu et al., 2016a), which is consistent with the experimental result.

**Fig. 4.** Effect of temperature on (a) the formation of THMs and (b) the formation of I-THMs ([free chlorine] = 2 mg L$^{-1}$; pH = 7; [Br$^-$] = 200 µg L$^{-1}$; [I] = 200 µg L$^{-1}$; [Trp] = 1 mg L$^{-1}$)

### 3.2.4 Effect of pH on the formation of THMs and I-THMs
Fig. 5 shows the effect of pH on the formation of THMs and I-THMs. The formation of THMs and I-THMs increased from 7.38 µg L$^{-1}$ and 8.68 µg L$^{-1}$ to 27.73 µg L$^{-1}$ and 30.41 µg L$^{-1}$, respectively, when pH increased from 5 to 9. It was indicated that the increase in pH would promote the formation of THMs and I-THMs. A similar result of the correlation between THMs or I-THMs and pH was obtained by Hu et al. (2010).

When pH was 5, CHBr$_2$I was the dominant I-THMs, which accounted for 56.5% of the total I-THMs. While pH increased from 6 to 9, CHI$_3$ accounted for 50.38%, 49.85%, 53.11% and 51.12%, respectively. It indicated that CHI$_3$ was the predominant, followed by CHBrI$_2$. The phenomena could be explained by the distribution of iodine and oxidant species. HOI is the dominant iodine species at neutral to nearly alkaline conditions, and I$_2$ is the dominant one at acidic conditions (Liu et al., 2016). As shown in Fig. S1, the distribution of HOCl in water decreases while the distribution of $\text{OCl}^-$ continuously with the increase in pH (Wu, 2005; Zhang et al., 2018). At neutral and alkaline conditions, the dissociation of HOCl to the less powerful $\text{OCl}^-$occurs (Criquet et al., 2012), which can oxidize less HOI to the stable $\text{IO}_3^-$. Therefore, more residual HOI can promote the incorporation of iodine to THMs and result in the increase in CHI$_3$ and CHBrI$_2$.

**Fig. 5.** Effect of pH on (a) the formation of THMs and (b) the formation of I-THMs ([free chlorine] = 2 mg L$^{-1}$; T = 25 °C; [Br$^-$] = 200 µg L$^{-1}$; [I] = 200 µg L$^{-1}$; [Trp] = 1 mg L$^{-1}$)

3.2.5 Effect of $\text{Br}^-/\text{I}^-$ ratio on the formation of THMs and I-THMs

Fig. 6 shows that $\text{Br}^-/\text{I}^-$ ratio significantly affects the formation of THMs and I-THMs. As shown in Fig. 6(a), with the increase in $\text{Br}^-/\text{I}^-$ ratio from 1:7 to 7:1, the concentration of THMs increased from 7.49 µg L$^{-1}$ to 33.82 µg L$^{-1}$. CHBr$_3$ was
increasing fastest from 1.03 µg L\(^{-1}\) to 13.63 µg L\(^{-1}\). As shown in Fig. 6(b), with the increase in \(\text{Br}/\Gamma\) ratio, the concentration of I-THMs significantly decreased from 38.11 µg L\(^{-1}\) to 5.38 µg L\(^{-1}\). The concentration of CHI\(_3\) decreased the most from 32.26 µg L\(^{-1}\) to 1.52 µg L\(^{-1}\). Recent research shows that the formation of CHI\(_3\) increases from 12.2 µg L\(^{-1}\) to 579.9 µg L\(^{-1}\), with the increase in \(\Gamma\) from 5 µM to 100 µM (Ye et al., 2013).

The increase in \(\text{Br}/\Gamma\) ratio means the increase in \(\text{Br}^-\) and the decrease in \(\Gamma^-\), which leads to the reduction of hypohalous acid to generate less I-THMs. HOBr formed through reactions between chlorine and \(\text{Br}^-\) can oxidize HOI to the stable \(\text{IO}_3^-\), which leads to the decrease in \(\Gamma^-\) incorporation into THM formation (Liu et al., 2018). Moreover, \(\text{Br}^-\) can be recycled by chlorine to HOBr, which leads to complete bromine incorporation into NOM (Langsa et al., 2017). According to a higher reactivity of HOBr toward NOM than that of HOCl, HOBr favored the formation of bromine-substituted THMs (Zhang et al., 2015). Hence, the increase in \(\text{Br}^-\) would promote the formation of bromine-containing THMs. Combining with the experiment results in the real water, the amount of I-THMs produced by Sample 1 was the least (as shown in Fig. 1(b)) because of the low \(\Gamma^-\) concentration level (as shown in Table S3). Therefore, in order to reduce the formation of THMs and I-THMs, the concentration of \(\text{Br}^-\) and \(\Gamma^-\) should be controlled at a low level.

**Fig. 6.** Effect of \(\text{Br}^-/\Gamma^-\) ratio on (a) the formation of THMs and (b) the formation of I-THMs ([free chlorine] = 2 mg L\(^{-1}\); \(T = 25^\circ\text{C}\); \(\text{pH} = 7\); [Trp] = 1 mg L\(^{-1}\))

3.3 The degradation pathway of Trp chlorination

According to the experimental results, the formation of THMs and I-THMs by Trp chlorination was great. Moreover, the previous literature shows that Trp has the
most chlorine consumption among all amino acids (Hong et al., 2009). Therefore, the
indole heterocycle of Trp was the main structure of chlorine consumption. To obtain
the degradation pathway of Trp, the intermediates formed during Trp chlorination
were measured by GC-MS.

3.3.1 The formation of THMs

Fig. S2 shows the atom potential map of Trp. It is seen from Fig. S2 that the atom
potential of C2 and C3 are 0.03 V and 0.6 V, respectively, so the difference in atom
potential between C2 and C3 is greater. Thus, the bond between C2 and C3 was firstly
cleaved and resulted in a loss of CO$_2$ from Trp to produce tryptamine by chlorine. The
atom potential of C4 and C7 are -0.06 V and -0.16 V, respectively. Then, because of
the stability of the five-membered heterocyclic ring and the benzene ring, the
potential difference between C4 and C7 was 0.1 V and the bond between C4 and C7
was cleaved to form ethylamine and 3-chloroindole. The five-membered heterocyclic
ring of 3-chloroindole had the property of electron donating, and the electron density
of the heterocyclic ring was higher than that of benzene ring. So, it was easier to
undergo electrophilic substitution reactions. The atom potential of C7 and C8 are
-0.16 V and -0.04 V, respectively. Then because of the large potential difference
between C7 and C8, and in the role of HOCl, the conjugated double bond (the bond
between C7 and C8) of the chloroindole group was cleaved to form a group which
contained trichloromethyl. Because of the strong negative inductive effect of
trichloromethyl, the C-C bond (the bone between C7 and C11) containing the
trichloromethyl group was cleaved to form TCM and benzamide. In the role of HOCl
and HOBr, benzamide occurred substitution reactions to form
N-(4-bromo-2-chlorophenyl) formamide (Fig. S3(a)). The m/z of
N-(4-bromo-2-chlorophenyl) formamide tested by GC-MS was 207, it was because of
the reactions shown in Fig. S4.

Tryptamine was also oxidized by HOCl to produce ethylamine. The ethylamine is a lower aliphatic amine containing primary amines, which react with HOCl to form chlorinated derivatives (Stanbro and Smith, 1979). According to the previous studies, under heating or alkaline conditions, the nitrogen atom is attacked by electrophiles, halogenated reagents, etc., and then the elimination reaction will be occurred by the primary amines to form the imine (Smith, P., 1965; Haslam, E., 1979; Fields, S. C. et al., 1995). Therefore, the amino group of the ethylamine in the experiments was chlorinated to form N-chloroamine. Under alkaline condition, the N-chloroamine removed a molecule of HCl to form C=N double bond and to produce the imine. The imine removed a molecule of NH$_3$ to form the acetaldehyde. Then, the acetaldehyde was oxidized by HOCl to form dichloroacetaldehyde (Fig. S3(b)) and chloral. Because of the instability of dichloroacetaldehyde and chloral in the solutions, they were finally decomposed into THMs (Fig. S3(c)) and the carboxylate. The proposed formation pathway of THMs by Trp chlorination is shown in Fig. 7a.

3.3.2 The formation of I-THMs

Because of the addition of $\Gamma^-$ to the solution, I-THMs were present during the reaction. The formation pathway of I-THMs by Trp chlorination was similar to that of THMs. As shown in Fig. S2, the potential difference between C2 and C3 is 0.57 V, which indicates that the bond between C2 and C3 is easily cleaved. First in the role of HOI, Trp removed a molecule of CO$_2$ to produce tryptamine. Second, because of the large potential difference between C4 and C7, the bonds between C4 and C7 were cleaved to form 3-iodoindole. Finally, in the role of HOI, the conjugated double bond (the bond between C7 and C8) of the iodoindole group was opened to form the benzamide and iodoform (Fig. S5). The proposed formation pathway of I-THMs by
Trp chlorination is shown in Fig. 7(b).

**Fig. 7.** The formation pathway of (a) THMs and (b) I-THMs by Trp chlorination (pH=7; [free chlorine] = 2 mg L\(^{-1}\); T = 25 °C; [Br\(^{-}\)] = 200 µg L\(^{-1}\); [I\(^{-}\)] = 200 µg L\(^{-1}\); [Trp] = 1 mg L\(^{-1}\)) (The circle represent the intermediates detected.)

3.4 Toxicity analysis of Trp chlorination

Fig. S6 shows that with the increase in reaction time, the inhibition of bioluminescence increased during Trp chlorination. The inhibition of bioluminescence significantly increased in the first 1 h, which indicated the formation of some toxic intermediates during Trp chlorination, mainly aromatic compounds (Wang et al., 2016). After 1 h, the constant increase in toxicity could be attributed to the formation of unique derivatives and halogenated DBPs such as THMs and I-THMs. From the previous experimental results (Fig. 2), the formation of THMs and I-THMs during Trp chlorination was stabilized at 24 h, which was consistent with the trend of toxicity. When the water sample was contacted with *Vibrio fischri* bacteria for 15 min, the inhibition of Trp chlorination was 60%. Therefore, the inhibition of bioluminescence was found to increase during Trp chlorination with the increase in time, and might be caused by the intermediate products.

4 Conclusions

1) The formations of THMs and I-THMs during Trp chlorination well fitted a new first-order reaction kinetic. Moreover, the formation of I-THMs was significantly more than that of THMs.

2) The formation of THMs increased with chlorine increased. While the formation of I-THMs exhibited an increasing phase then decreasing phase with the increase of chlorine, reaching a maximum yield at 2 mg L\(^{-1}\) as Cl\(_2\).

3) The formation of THMs and I-THMs increased with the increase in temperature.
The increase in temperature would promote the reaction and enhance the formation of THMs and I-THMs.

4) The increase in pH would promote the formation of THMs and I-THMs. The formation of THMs and I-THMs increased faster under acidic conditions but slower under alkaline conditions.

5) With the ratio of Br/T increased, the formation of THMs increased and the formation of I-THMs decreased.

6) The reaction between Trp and chlorine could open the ring of pyrrole and then were further halogenated to form THMs and I-THMs. The ethylamine was also generated during Try chlorination and then formed intermediate products of imine by reacting with HOCl, which could be further halogenated to THMs.

7) With the increase in time, the inhibition of bioluminescence was found to increase during Trp chlorination, and might be caused by the intermediate products.

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Fig. 1. The formation of (a) THMs and (b) I-THMs by chlorination of five samples ([free chlorine] = 2 mg L\(^{-1}\); T = 25 °C)
Fig. 2. The generated curves of (a) THMs and (b) I-THMs by Trp chlorination ([free chlorine] = 2 mg L$^{-1}$; $T = 25$ °C; pH = 7; [Br$^{-}$] = 200 µg L$^{-1}$; [I$^{-}$] = 200 µg L$^{-1}$; [Trp] = 1 mg L$^{-1}$)
Fig. 3. Effect of the dosage of chlorine on (a) the formation of THMs and (b) the formation of I-THMs \((T = 25 \, ^\circ\text{C}; \, \text{pH} = 7; \, [\text{Br}^-] = 200 \, \mu\text{g L}^{-1}; \, [\text{I}] = 200 \, \mu\text{g L}^{-1}; \, [\text{Trp}] = 1 \, \text{mg L}^{-1})\)
Fig. 4. Effect of temperature on (a) the formation of THMs and (b) the formation of I-THMs ([free chlorine] = 2 mg L$^{-1}$; pH = 7; [Br$^-$] = 200 µg L$^{-1}$; [I$^-]$ = 200 µg L$^{-1}$; [Trp] = 1 mg L$^{-1}$).
Fig. 5. Effect of pH on (a) the formation of THMs and (b) the formation of I-THMs ([free chlorine] = 2 mg L\(^{-1}\); T = 25 °C; [Br\(^-\)] = 200 µg L\(^{-1}\); [I\(^-\)] = 200 µg L\(^{-1}\); [Trp] = 1 mg L\(^{-1}\))
Fig. 6. Effect of $\text{Br}^{-}/\text{I}^{-}$ ratio on (a) the formation of THMs and (b) the formation of I-THMs ($[\text{free chlorine}]=2 \text{ mg L}^{-1}; T=25 ^\circ\text{C}; \text{pH}=7; [\text{Trp}]=1 \text{ mg L}^{-1}$)
Fig. 7. The formation pathway of (a) THMs and (b) I-THMs by Trp chlorination (pH = 7; [free chlorine] = 2 mg L\(^{-1}\); T = 25 °C; [Br\(^{-}\)] = 200 µg L\(^{-1}\); [I\(^{-}\)] = 200 µg L\(^{-1}\); [Trp] = 1 mg L\(^{-1}\)) (The circles represent the intermediates detected.)
Highlights

- The tryptophan (Trp) chlorination fitted a new first-order kinetic model.
- The formation of trihalomethanes (THMs) and iodinated trihalomethanes (I-THMs) increased with the increase in temperature.
- The toxicity of water sample during Trp chlorination was increasing.
- The reaction between Trp and chlorine could open the ring of pyrrole and then were further halogenated to form THMs and I-THMs.