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RESEARCH ARTICLE

Factorial analysis of the trihalomethane formation in the reaction of colloidal, hydrophobic, and transphilic fractions of DOM with free chlorine

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Abstract
Background, aim, and scope This study focuses on the factors that affect trihalomethane (THMs) formation when dissolved organic matter (DOM) fractions (colloidal, hydrophobic, and transphilic fractions) in aqueous solutions were disinfected with chlorine.
Materials and methods DOM fractions were isolated and fractionated from filtered lake water and were characterized by elemental analysis. The investigation involved a screening Placket-Burman factorial analysis design of five factors (DOM concentration, chlorine dose, temperature, pH, and bromide concentration) and a Box-Behnken design for a detailed assessment of the three most important factor effects (DOM concentration, chlorine dose, and temperature).
Results The results showed that colloidal fraction has a relatively low contribution to THM formation; transphilic fraction was responsible for about 50% of the chloroform generation, and the hydrophobic fraction was the most important to the brominated THM formation.
Discussion When colloidal and hydrophobic fraction solutions were disinfected, the most significant factors were the following: higher DOM fraction concentration led to higher THM concentration, an increase of pH corresponded to higher concentration levels of chloroform and reduced bromoform, higher levels of chlorine dose and temperature produced a rise in the total THM formation, especially of the chlorinated THMs; higher bromide concentration generates higher concentrations of brominated THMs. Moreover, linear models were implemented and response surface plots were obtained for the four THM concentrations and their total sum in the disinfection solution as a function of the DOM concentration, chlorine dose, and temperature. Overall, results indicated that THM formation models were very complex due to individual factor effects and significant interactions among the factors.
Conclusions In order to reduce the concentration of THMs in drinking water, DOM concentrations must be reduced in the water prior to the disinfection. Fractionation of DOM, together with an elemental analysis of the fractions, is important issue in the revealing of the quality and quantity characteristics of DOM. Systematic study composed from DOM fraction investigation and factorial analysis of the responsible parameters in the THM formation reaction can, after an evaluation of the adjustment of the models with the reality, serves well for the evaluation of the spatial and temporal variability in the THM formation in dependence of DOM. However, taking into consideration the natural complexity of DOM, different operations and a strict control of them (like coagulation/flocculation and filtration)

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has to be used to quantitatively remove DOM from the raw water.

Recommendations and perspectives Assuming that this study represents a local case study, similar experiments can be easily applied and will supply with relevant information every local water treatment plant meeting problems with THM formation. The coagulation/flocculation and the filtration stages are the main mechanisms to remove DOM, particularly the colloidal DOM fraction. With the objective to minimize THMs generation, different unit operation designed to quantitatively remove DOM from water must be optimized.

Keywords Factorial analysis · Response surface methodology · Chlorine water disinfection · Colloidal · Hydrophobic · Transphilic · Trihalomethanes formation · Disinfection by products

1 Background, aim, and scope

Since the first study conducted by Rook in 1974, it has been established that the use of chlorine for disinfecting drinking water leads to the formation of various disinfection byproducts (DBPs) potentially harmful for human health (Bellar et al. 1974; Nieuwenhuijsen et al. 2000; Chang and Young 2000; Richardson and Thruston 2003; Richardson et al. 2007). Regardless, chlorine remains the most commonly used disinfectant because it is effective, relatively inexpensive and has a disinfection residual property, which is important to prevent possible sources of contamination in the distribution system. Among the DBP groups identified in chlorinated water (Hrudey 2009), only the trihalomethane (THM) family is regulated by European Community legislation, which includes chloroform, bromodichloromethane, chlorodibromomethane, and bromoform (Council Directive 98/83/EC). THMs constitute an important matter of public health concern, since they are regarded as carcinogens and, more recently, epidemiological studies indicate that they are also associated with reproductive and developmental problems (McGeehin et al. 1993; Simpson and Hayes 1998; Lewis et al. 2006; Savitz et al. 2006). However, many epidemiologists and other scientists contest many of epidemiological studies involving DBPs in drinking water, particularly those involving acute exposure, which suffer from the misclassification of exposure (Reif et al. 1996). Actually, a review of epidemiological studies about cancer risks found only a somewhat consistent association among chlorinated surface waters and bladder cancer. Also, weak to moderate bladder cancer risks were found associated with long-term exposure to chlorinated surface water and THM (Villanueva et al. 2007; Hamidin et al. 2008). The health risk concern from exposure to THM forced the European Union to establish a new drinking water quality regulation that changed the maximum levels of total THM (TTHM, the sum of all individual trihalomethanes) allowed in drinking water from 150 μg L^{-1} to 100 μg L^{-1} (Council Directive 1998). However, to strictly follow this directive and apply the practices in municipal treatment plants that supply safe and potable water, understanding the process of THM formation is crucial.

Natural organic matter dissolved in water (DOM) is usually considered the precursor of DBP (von Gunten et al. 2001; Rostad et al. 2000; Leehneer et al. 2001; Panyapinyopol et al. 2005). DOM is a complex mixture of various compounds with very different chemical properties. Many efforts have been made to characterize DOM in order to improve its removal and reduce DBP formation during water disinfection (Croué et al. 1999; Croué 2004). The most common practice for the isolation and fractionation of DOM from water is using XAD resins and ion-exchange resins (Leenheer et al. 2000; Leenheer 2004). Recently, Leenheer (2004) proposed an operational scheme to separate DOM into four fractions: colloidal, hydrophobic, transphilic, and hydrophilic. A number of studies have attempted to correlate some specific characteristics of organic matter, functionality, and aromaticity with THM formation (Norwood et al. 1980; Gallard and von Gunten 2002; Dickenson et al. 2008). Likewise, many investigations have focused on operational parameters such as chlorine dose, water temperature, pH, and reaction time, which are regarded as influential for THM formation (Peters et al. 1980; Radiq and Rodriguez 2004). In addition, the bromide ion in raw water may also play an important role in the THM formation reaction, leading to a predominance of brominated THMs (Xue et al. 2008; Nikolaou 2004). As a result of the intense research in this area, during the last years, many mathematical models have been developed for predicting DBP and THM formations (Sohn et al. 2004; Platikanov et al. 2007). These models mainly focused on the prediction of total THM or chloroform formation. In spite of the large number of studies examining DBP formation of the isolated DOM fractions from different water sources by chlorination in different conditions, there are still contradictory results, mainly in the disinfectant dosage and pH effect (Nikolaou 2004; Lu et al. 2009).

The aim of this study was to utilize a method (Rodrigues et al. 2007) that has been proposed to determine the factors that affect the formation of the four THMs by chlorine disinfection of different DOM fractions (hydrophobic, colloidal, and transphilic) in a prototype laboratory simulation. DOM fractions were extracted from water samples of the Caldeirão dam (Guarda, Portugal) by a reverse osmosis water pre-concentration procedure, followed by dialysis and adsorption resins (Leenheer and Croué 2003).

THM formation is a complex process that depends on several factors and usually involves interactions of those factors. This study uses a factorial analysis strategy in order to identify the most THM formation-relevant factors and the way they influence THM formation (Rodrigues et al. 2007; Esteves da Silva et al. 2001). Two experimental designs, based on a Placket-Burman design of five factors (DOM fraction, chlorine dose, temperature, pH, and bromide ion concentration) and a Box-Behnken design for the analysis of three factors (DOM fraction concentration, chlorine dose, and temperature), were used to identify the most important factors in the formation of the four THM species and in the calculation of the corresponding response surfaces. A Box-Behnken design was chosen because it enabled a more precise study of the effect of several factors, as well as to obtain response surfaces with a relatively few number of experiments and with only three levels for each of the factors under analysis.

2 Materials and methods

As before mentioned, DOM fractions were obtained from Caldeirão dam in Guarda, Portugal. To be brief, a known volume of water (about 200 L) was concentrated using a reverse osmosis system. This system consisted of an electric pump, ionic exchange resins, and a reverse osmosis membrane. The concentrated water collected after the osmosis process was filtered using 0.45 μm Whatman cellulose acetate membranes and acidified to pH 2 with 6 M hydrochloric acid.

DOM fraction isolation was carried out in several stages: (1) deposition of the concentrated water solution, acidified to pH 1, in a dialysis bag (Spectrum, Spectra/Per), with a 3.5 kDa cutoff, (2) immersion during 36 h (three times 4 L) in a 0.1 M HCl solution (Merck); (3) immersion of the dialysis bag in 0.2 M HF solution followed by immersion of the dialysis bag in deionized water, The dialysis bag retained the colloid fraction, which was frozen and lyophilized, and (4) the dialyzed solution was sequentially eluted by XAD-8 (Fluka) and XAD-4 (Sigma) column, which adsorbed the hydrophobic (HPOF) and transphilic fractions, respectively. The HPOF and transphilic fraction, adsorbed onto XAD-8 and XAD-4 columns, respectively, were then eluted with a mixture of acetonitrile (Merck) and water in a proportion of 75% and 25%, respectively (Fig. 1). The solutions with the fractions were frozen and lyophilized (B. Braun, Christ LDC-1). Fourier transform infrared (FT-IR) spectra of the DOM fractions were done with a Bruker, vector 22 model, FTIR spectrophotometer.

THM ($CHCl_3$, chloroform; $CHBrCl_2$, bromodichloromethane; $CHBr_2Cl$, dibromochloromethane; and $CHBr_3$, bromoform) 200 g L^{-1} standard solution in methanol (SUPELCO, Bellefonte, USA) was used for the preparation of the aqueous standard solutions in the μg L^{-1} range (0.5–30 μg L^{-1}). All reagents were of analytical grade quality. The sodium hypochlorite used was a commercial solution.

2.1 Laboratory simulation of a water disinfection process

The disinfection process of the water sample containing DOM followed the following steps: we (a) placed a reaction vessel of 250 ml volume, with an aqueous solution of DOM (concentrations of 0.5, 2.7, and 5 mg L^{-1}), in a water bath at a constant temperature; (b) added to the DOM fraction solution a volume of sodium chloride to achieve a final concentration of 10 mg L^{-1} of chloride anion and a predetermined volume of potassium bromide (final concentration of 0.1, 0.55, and 1.0 mg L^{-1}); (c) adjusted pH with hydrochloric acid and/or sodium hydroxide to predetermined values (pH 6.0, 7.0, and 8.0); (d) added a predetermined amount of sodium hypochlorite to begin the disinfection reactions; (e) kept the sample at a constant temperature in a water bath; (f) 20.00 mL were removed at times zero (after sodium hypochlorite addition), 5 and 30 min to perform the THM analysis (after sample

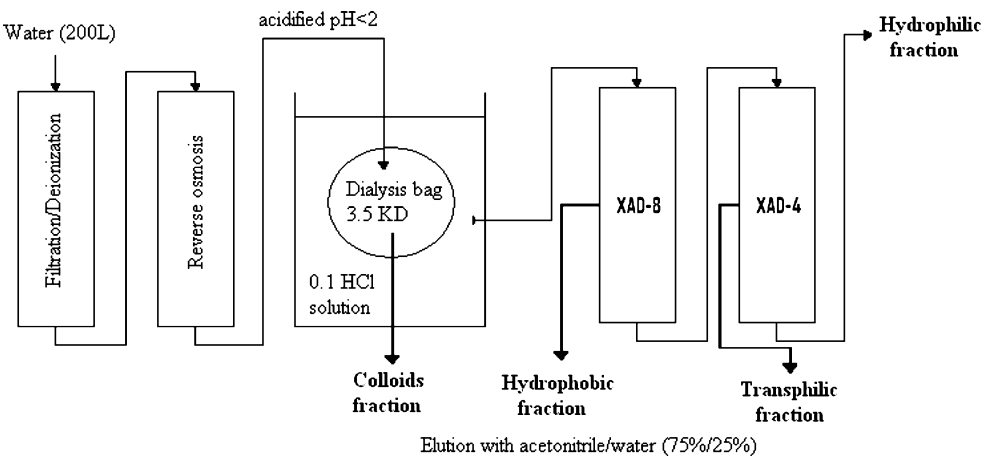

Fig. 1 Isolation protocol for colloids, hydrophobic, and transphilic fractions from water

collection, 30 μL of a solution 2 M sodium thiosulphate were added to eliminate free chlorine); (g) free chlorine was analyzed in all samples using a portable photometer kit (ELE International Limited, England).

THMs were analyzed by gas chromatograph-electron capture detector (GC-ECD). Gas chromatographic analyses were performed with a Chrompack CP9003 GC gas chromatograph equipped with a ^{63}Ni electron capture detector and a split/splitless injector. The column used was a Chrompack CP-Sil 13CB (25 m×32 mm, 1.2 μm) fused-silica column. Headspace analysis and GC-ECD parameters are shown in (Rodrigues et al. 2007). The limits of detection for the four THM of the HS-GC-ECD were in the range 0.3-1.4 μg L^{-1} and were calculated using the following criteria: $\mathrm{LOD} = (a + 3\mathrm{Sy}/x)$, where a is the intercept of the calibration curve and Sy/x is the random error in the y direction (Miller and Miller 2000).

2.2 Organization of the study

THM formation study was organized using two different experimental designs. First, a preliminary screening analysis was performed following a Placket-Burman design, focusing on the effect's evaluation of the five main factors: DOM fraction concentration, chlorine dose, pH, water temperature, and bromide anion concentration. Second, this preliminary study was followed using a Box-Behnken experimental design to estimate the effects of the two factors that have a natural variability (DOM concentration and water temperature) and one operationally controlled factor (chlorine dose) in the water treatment plant.

Table 1 shows concentrations and volumes of the factors under investigation, which were used for the preparation of simulated disinfection experiments. The DOM, chloride and bromide concentrations, and temperature were chosen to represent the natural variation of these parameters along the year in waters of the Caldeirão Dam. The concentrations of sodium hypochlorite were chosen such that there was always an excess of free chlorine. The minimum concentration of free chlorine (0.4 mg L^{-1}) led to free residual chlorine of 0.01 mg L^{-1} at the end of the experiment and the maximum concentration of free chlorine (2.4 mg L^{-1}) led to free residual chlorine of 0.1 mg L^{-1}. These values of the free residual chlorine did not decrease markedly after 60 min of subsequent reaction (the experimental time was about 90 min). This is in agreement with the fact of most THM growth rate was higher during the first 69–90 min (Korshin et al. 2002; Fabbricino and Korshin 2005; Fabbricino and Korshin 2009).

All calculations and data analysis were done using peak areas obtained from the recorded chromatogram using Chrompack CP-Maitre I/II software (version 2.5). The experimental design formulation and the corresponding analysis of the effects (ANOVA) and response surface calculations were done using The Unscramble v9.2 (CAMO PROCESS AS, Oslo, Norway).

3 Results

3.1 Characterization of the DOM fractions

To obtain information about the chemical structure of the investigated fractions and to relate it to the THM formation afterwards, an elemental analysis and FT-IR spectroscopy was performed. Elemental analysis and the H/C and the C/N atomic ratios of the three DOM fractions are presented in Table 2. The analysis of this table shows that the main differences among the three DOM fractions are the following: (1) higher elemental percentages of nitrogen and sulfur are detected in the colloidal fraction; (2) the H/C ratios in the HPOF fraction are lower than that in the others; (3) C/N ratio increases according to this order: colloidal fraction (lowest), transphilic fraction (middle) and HPOF (highest). Similar trends were observed for fractions analyzed by Leenheer and others (2000). These results show that the colloidal fraction is characterized with higher amounts of protein residuals in their molecules and a lesser

Table 1 Experimental designs, factors, and corresponding levels

Factors	Levels		
Placket-Burman design (8 + 3 center experiments)[a]			
DOM fraction concentration in mg L^{-1}	0.5	2.75	5
Bromide anion concentration (Br^-) in mg L^{-1}	0.1	0.55	1.00
pH	6.0	7.0	8.0
Water temperature (T) in °C	10	17.5	25
Chlorine (Cl_2) in mg L^{-1}	0.4	1.4	2.4
Box-Behnken design (12 + 3 center experiments)[a,b]			
DOM fraction concentration in mg L^{-1}	0.5	2.75	5
Water temperature (T) in °C	10	17.5	25
Chlorine (Cl) in mg L^{-1}	0.4	1.4	2.4

[a] A constant background concentration of 10 mg L^{-1} chloride anion was used in all experiments

[b] A constant background concentration of 10 mg L^{-1} chloride and 0.1 mg L^{-1} bromide anions were used in all experiments

Table 2 Elemental composition (mass %) and atomic DOM fractions ratios

DOM fraction	N	C	H	S	H/C	C/N
Colloidal	4.3	40.2	5.6	0.9	1.7	10.9
Transphilic	3.1	54.4	6.6	<0.3	1.5	20.5
Hydrophobic	1.3	56.2	5.9	0.7	1.3	50.4

amount of carbohydrate structures. However, the HPOF is dominated more with condensed aromatic structures (Leenheer et al. 2000; Leenheer 2004). The transphilic fraction takes intermediate position, possessing lower amounts of protein structures than the colloids and lower amounts of condensed aromatics than the HPOF.

IR spectra of the three fractions are shown in Fig. 2. The band at about 3,300 cm^{-1} is generally attributed to OH groups and bands at 2,900–2,930 cm^{-1} are assigned to CH, CH_2, and CH_3 stretching of the aliphatic groups. The bands at 1,640–1,680 cm^{-1} and 1,560–1,551 cm^{-1} are attributed to CO stretching vibration of carboxylic acids and ketones/quinones, respectively. The bands at about 1,450 cm^{-1} and 1,410 cm^{-1} are attributed to CH deformation of aliphatic and CH_3 groups, respectively. Also, bands in the 1,280–1,137 cm^{-1} regions are attributed to CO stretching of esters, ethers and phenols, and the band at about 830 cm^{-1} can be assigned to OH stretching vibration of carboxylic groups. In the IR spectrum of the colloidal fraction, the band located at 1,050 cm^{-1} due to CO groups is particularly important because these groups are indicative of the presence of N-acetylglucosamine (Croué 2004), formed from the oxidation of carbohydrates with amino groups from the bacterial cell wall structure (Hwang et al. 2001; Leenheer 2004). In the case of hydrophobic and transphilic fractions, there is a strong intensity band near 1,720 cm^{-1} which suggests a relatively greater abundance of carbonyl groups.

3.2 Qualitative analysis of factor effects using a Placket-Burman design

Table 1 shows the factors and levels used for the evaluation of the main effects on THM formation. The five parameters were studied using a Placket-Burman design (eight plus three center experiments). In this screening analysis, only the HPOF and colloid fractions were studied because the available quantity of transphilic fraction was very low. Table 3 shows the analysis of the effects of the five parameters on the four individual THMs and their total sum using Placket-Burman design experiments. The experimental error was estimated using replicated center samples.

Some results from the analysis in Table 3:

1. Higher the concentration of the colloidal and hydrophobic fraction greater the total THM production. This fact was expected because DOM concentration is the main precursor from which THM originates (Leenheer 2004; Lu et al. 2009). HPOF concentration was a very significant parameter for the formation of multi-chlorinated trihalomethanes, whereas colloidal fraction was more influential in the formation of mixed bromochloromethanes. Bromoform formation did not show any significant dependence on the two fraction concentrations.
2. pH positively affects the formation of $CHCl_3$ and $CHBrCl_2$ and slightly affects the formation of $CHBr_2Cl$ for both DOM fractions. In general, a pH increase (above pH 7) resulted in a reduction in the concentration of the brominated species and an increase in the concentration of $CHCl_3$. This occurs, possibly, because the formation of the hypochlorite ion ($Cl_2 + OH^- \rightleftharpoons OCl^- + Cl^- + H^+$ or $Cl_2 + H_2O \rightleftharpoons HOCl + Cl^- + H^+$ and $HOCl \rightleftharpoons OCl + H^+$) is shifted to the right with increasing pH (i.e., increasing OH^-). Consequently, hypochlorite ion concentration increases, leading to

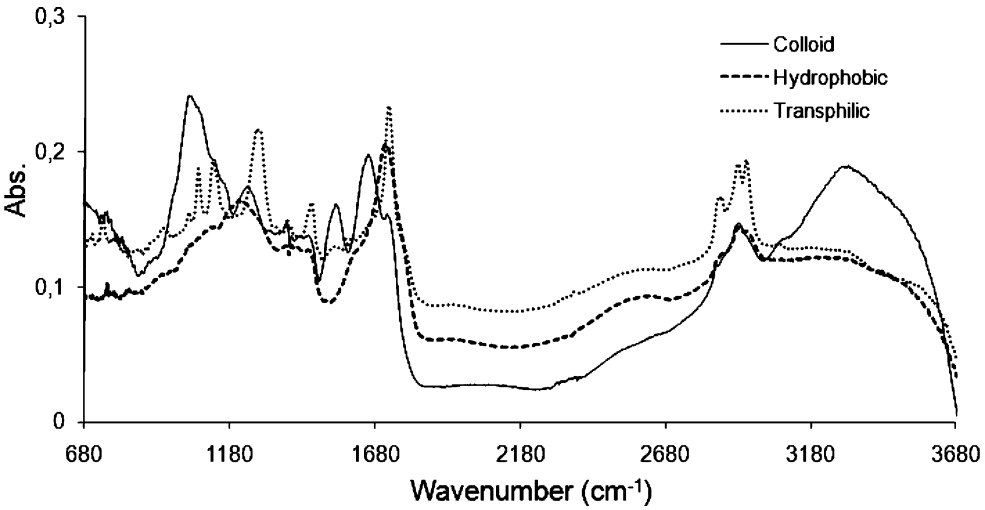

Fig. 2 FT-IR spectra of colloid, hydrophobic, and transphilic fractions

Table 3 Qualitative analysis of the effects of the five parameters on the four THM and TTHM for HPOF and Colloidal fractions

Factor	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$	TTHM
HPOF fraction					
pH	+	+	NS	NS	+
Chlorine	++	++	+	NS	+++
Temperature	NS	NS	NS	NS	NS
Bromide concentration	−	−	NS	+	−
HPOF concentration	+++	+++	+	NS	+++
Colloidal fraction					
pH	++	+++	++	NS	+
Chlorine	++	+++	−	NS	+
Temperature	++	++	++	NS	+
Bromide concentration	−	−	+++	+	NS
Colloids concentration	++	+++	+++	NS	++

NS not significant factor; + means a positive effect, − means negative effect. *More than one* + *or* − signs mean stronger effects

predominance of chlorinated species (Nikolaou 2004). Also, the decreasing concentration of brominated THMs at pH values above 7–8 may be due to the following disproportionate reaction of the hypobromite ion at basic pH values (Bard et al. 1985): $OBr^- + 2HOBr \rightarrow BrO_3^- + 2Br^- + 2H^+$. From this equation, the OBr^- is disproportionate to bromate and bromide ions, neither of which reacts to organic matter. Chlorination is a typical electrophilic substitution which occurs in many steps, for example, in phenol groups the H^+ is release from phenolic ring to the solution. Thus, pH would affect the equilibrium of the reaction. The effects of pH on chlorination process must be explained simultaneously by the deprotonation of hypochlorite and/or the organic compound which may change the reaction kinetic (Westerhoff et al. 2004; Ge et al. 2006).

3. Similar behavior was observed for the chlorine dose used in the disinfection. The higher dose of Cl_2 generates higher concentrations of TTHM, especially $CHCl_3$ and $CHBrCl_2$. This is also expected since higher chlorine doses lead to an increase of hypochlorite ion concentration, as found in previous results in the literature (Rook 1974; Sohn et al. 2004).
4. Increasing the temperature produces an increase in the concentration of $CHCl_3$ and of mixed bromochloromethanes when the colloidal fraction is oxidized. The temperature parameter however does not have a significant effect during the chlorination of HPOF.
5. Bromide concentration produces a similar effect in THM formation of the two DOM fractions. High concentrations of bromide produce high concentrations of brominated THMs and relatively low concentrations of $CHCl_3$ and $CHBrCl_2$. It is well-known that $CHCl_3$ is formed in the reaction of DOM with OCl^- and $CHBr_3$ with OBr^-, and the amounts of $CHCl_3$ and $CHBr_3$ depend on the concentration of OCl^- and OBr^-, respectively. Higher concentrations of OBr^- are present in the case of higher concentration of bromide anion, resulting in the formation of a higher concentration of $CHBr_3$. As the concentration of OBr^- increases, the amount of $CHCl_3$ will decrease in response.

3.3 Preliminary analysis of the effect of the DOM fraction on the THM formation

Box-Behnken design analyses where performed to investigate the effect of DOM concentration, chlorine dose and temperature factors on THM formation. Table 1 shows the levels of these three factors under analysis. In this analysis, bromide concentration was kept constant at 0.1 mg L^{-1} since the natural water from the Caldeirão Dam has low concentrations of it due to an absence of geological or anthropological sources of bromide ions. Also, pH was kept constant at 7.0 because this is a common operational procedure implemented in water treatment plants. In spite of temperature parameter in Placket-Burman design was not a significant factor in the formation of THM in the HPOF fraction, we consider this in the Box-Behnken design because it may be important in the formation of THM in the other fractions and because this parameter have a great variability in water treatment plant along year seasons.

As shown in Table 4, the amount of generated THMs in the experiment was characterized by a rather large range in

Table 4 Concentration (μg L^{-1}) ranges of the four THM and total THM generated from the disinfection of aqueous solutions of the three DOM fractions

THM	Colloidal	HPOH	Transphilic
$CHCl_3$	3.3–6.0	1.6–4.6	4.0–21.3
$CHBrCl_2$	3.5–4.5	3.6–11.0	37–8.0
$CHBr_2Cl$	4.0–5.4	4.3–15.0	4.4–13.1
$CHBr_3$	3.2–7.1	5.1–12.4	3.2–15.7
TTHM	14.8–22.2	15.0–42.9	17.7–39.6

concentration. This results show the relevance of this three factors under investigation for THM generation. Figure 3a shows the pie plots with the percentage contributions of the three DOM fractions in the production of the four THM. A preliminary analysis of Fig. 3a shows the following trends: (1) the transphilic fraction is responsible for the production of about half the amount of chloroform, followed by the colloids and HPOF; (2) there is an increase in the percentage of the most brominated THM in the HPOF and transphilic DOM fractions and there is a decrease in their percentages in the colloidal DOM fraction.

Figure 3b shows the pie plot with the percentage contribution in the three DOM fractions for the total production of THM. This plot suggests the following trend in the order of total THM production: transphilic > hydrophobic > colloidal. These results are in agreement with the conclusions of Marhaba et al. (2006), where the differences of DBPs yields between the fractions are possibly due to their different characteristics of functional groups and structures. Indeed, the colloidal fraction shows a lower amount of aromatic and polyphenolic compounds than the transphilic and hydrophobic fraction, which can explain a greater THM generation by the last two fractions.

3.4 Response surface analysis in the formation of the four individual THMs

ANOVA of factor effects in the formation of individual THMs was done as well and linear models were obtained (data not shown). Included in the models where the coefficients of the factors that were statistically significant at the 5% level, as well as coefficients with absolute values higher than the corresponding standard deviations.

Colloidal fraction models

$$\mathrm{CHCl_3}\left(\mu\mathrm{gL^{-1}}\right) = 4.8 + 0.2\,\mathrm{colloids} + 0.2\mathrm{T} \times \mathrm{Cl} - 0.25\ \mathrm{colloids} \times \mathrm{T} + 0.2\,\mathrm{Cl}^2$$

$$\mathrm{CHBrCl_2}\left(\mu\mathrm{gL^{-1}}\right) = 3.7 + 0.03\,\mathrm{colloids} + 0.01\,\mathrm{T} + 0.01\mathrm{Cl} + 0.1\,\mathrm{T} \times \mathrm{Cl} + 0.1\,\mathrm{Cl}^2 - 0.06\ \mathrm{colloids}^2$$

$$\mathrm{CHBr_2Cl}\left(\mu\mathrm{gL^{-1}}\right) = 4.6 + 0.005\,\mathrm{T} + 0.02\,\mathrm{Cl} + 0.1\ \mathrm{colloids} - 0.04\,\mathrm{T} \times \mathrm{Cl} - 0.03\,\mathrm{T} \times \ \mathrm{colloids} + 0.3\,\mathrm{Cl} \times \mathrm{colloids} + 0.06\,\mathrm{Cl}^2 - 0.1\ \mathrm{colloids}^2$$

$$\mathrm{CHBr_3}\left(\mu\mathrm{gL^{-1}}\right) = 3.5 + 0.05\,\mathrm{Cl} + 0.3\,\mathrm{colloids} + 0.6\,\mathrm{Cl} \times \mathrm{colloids}$$

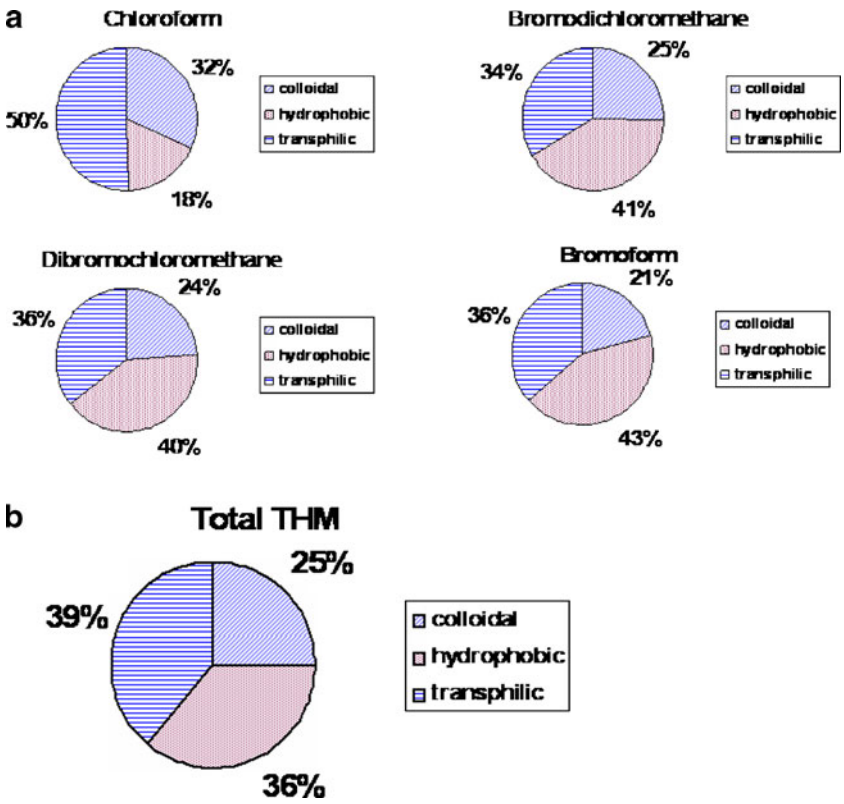

Fig. 3 Percentages of the four THM (**a**) and TTHM (**b**) generated from the three DOM fractions

The three factors under investigation play significant roles in a quite complex THM generation. The results show that the amount of chlorine positively affects the formation of the four THMs.

Increasing the colloid concentration leads to a decrease in the production of mixed chloro-bromomethanes, but produces a slight increase in the production of chloroform and a strong increase in the bromoform formation. This observation is contrary to the Placket-Burman screening analysis that the colloidal fraction is more influential in the formation of mixed bromochloromethanes than bromoform. This erroneous result is a consequence of a lack of degrees of freedom of the screening design which results in an unreliable detailed factorial analysis as consequence of the mixing effect of the factors. This erroneous result must be solved in the future by doing more experimental analysis in the same conditions and under less variable factors.

The temperature factor plays a controversial role. It has a strong independent effect and also interacts with chlorine in the production of $CHBrCl_2$. It has a positive influence as an independent factor, but the interaction with the chlorine produces the opposite effect on the formation of $CHBr_2Cl$. The role of the global temperature balance is therefore not clear in the formation of chloroform and bromoform during the colloid fraction chlorination.

HPOF fraction models

$$CHCl_3\left(\mu gL^{-1}\right) = 2.8 + 0.02\,T + 0.01\,Cl + 0.5\,HPOF + 0.1\,T \times HPOF - 0.1\,Cl^2 + 0.1\,HPOF^2$$
$$CHBrCl_2\left(\mu gL^{-1}\right) = 6.4 + 0.1\,T + 0.1\,Cl + 1\,HPOF + 0.3\,T \times HPOF + 0.3\,Cl \times HPOF - 0.5\,Cl^2$$
$$CHBr_2Cl\left(\mu gL^{-1}\right) = 9 + 0.1\,T + 0.1\,Cl + 1.4\,HPOF + 0.6\,T \times HPOF + 0.5\,Cl \times HPOF - 1.2\,Cl^2$$
$$CHBr_3\left(\mu gL^{-1}\right) = 10.7 + 0.1\,T + 0.1\,Cl + 1.1\,HPOF + 0.4\,Cl \times HPOF - 0.6\,T^2 - 1.2\,Cl^2 - 0.7\,HPOF^2$$

A consistency can be found in all four models: any excess of the amount of added chlorine generally leads to an increase in THM concentration. The increase of HPOF concentration independently, or when HPOF concentration interacts with the other two factors, increases the formation of $CHCl_3$, $CHBrCl_2$ and $CHBr_2Cl$. An interesting result is that high levels of HPOF fraction concentration, chlorine dose and temperature will reduce the formation of $CHBr_3$. These surprising results can be explained by the smaller bromide ions concentration, available in solution, and not by the HPOF concentration (Marhaba et al. 2006).

Also worth noting is that a positive interaction exists between HPOF fraction concentration and chlorine for the formation of brominated THMs. Moreover, in the disinfection of HPOF, it should be mentioned that temperature positively affects the production of chloroform and mixed chloro-bromomethanes.

Transphilic fraction models

$$CHCl_3\left(\mu gL^{-1}\right) = 6.1 - 0.4\,T + 0.1\,Cl - 1.8\,T \times Cl + 1.7\,Cl^2$$
$$CHBrCl_2\left(\mu gL^{-1}\right) = 4.8 + 0.04\,T + 0.1\,Cl + 0.5\,transphilic + 0.4\,T \times transphilic + 0.5\,Cl \times transphilic$$
$$CHBr_2Cl\left(\mu gL^{-1}\right) = 8 + 0.1\,T + 0.1\,Cl + 0.9\,transphilic + 0.6\,T \times transphilic + 1\,Cl \times transphilic - 0.7\,T^2$$
$$CHBr_3\left(\mu gL^{-1}\right) = 8.7 + 0.3\,T + 1\,transphilic + 1.6\,T \times Cl + 1.4\,T \times transphilic - 1.2\,Cl^2 - 1\,transphilic^2$$

Analysis of the three models for the formation of $CHCl_3$ shows different trends. The disinfection of colloidal and HPOF fractions derived very complex models. Controversially, the obtained model for the transphilic fraction chlorination depends only on chlorine dose and temperature and not on DOM concentration.

Another difference, compared to the other two experiments, is the interaction of the transphilic concentration with the other two factors (Cl and T) in the formation of brominated trihalomethanes.

In general, the global analysis of the DOM fraction disinfection reveals a high model complexity, i.e., many factor interactions were involved. Some models show significant lack of fit. Nevertheless, this result may be due to the relatively high precision of the THM measurements comparatively to a less precision in the control of operational factors, like kinetic time reaction, in the experimental procedures (Rodrigues et al. 2007). Even if we accept the existence of model misadjustment, factor effects on the THM formation are still realistic. A common feature to all DOM fractions is that the

highest values of all factors are responsible for the higher concentration of all THMs. Some exceptions to the above mentioned fact can be noticed for $CHCl_3$ formation, when transphilic fraction solution is disinfected. Relatively high values of the three factors cause the disinfection of HPOF and transphilic fractions solutions to form higher concentrations of $CHBr_3$. In contrast, this fact was not valid when colloids were oxidized. Also, relatively similar THM concentrations are formed when HPOF is disinfected and the factor levels are at a similar degree. Globally reducing the amount of HPOF and transphilic concentrations, together with keeping the temperature low, will yield a lower concentration of brominated THMs.

3.5 Response surface analysis of TTHM formation

Since EU regulation considers the sum of all individual THMs, the effects of DOM concentration, chlorine dose and temperature on the total THM formation was analyzed. ANOVA of factor effects for the formation of TTHM was calculated and linear models were obtained as well as response surfaces (Fig. 4).

$$\text{Colloidal fraction model}: \text{TTHM}\left(\mu\text{gL}^{-1}\right) = 16.6 + 0.06\,\text{T} + 0.1\,\text{Cl} + 0.65\ \text{colloids} + 0.3\,\text{T} \times \text{Cl} + \text{colloids} \times \text{Cl} + 0.6\,\text{Cl}^2$$

$$\text{HPOF fraction model}: \text{TTHM}\left(\mu\text{gL}^{-1}\right) = 28.9 + 0.3\,\text{T} + 0.3\,\text{Cl} + 4\,\text{HPOF} + 1.2\,\text{T} \times \text{HPOF} + 1.2\,\text{Cl} \times \text{HPOF} - 3\,\text{Cl}^2$$

$$\text{Transphilic fraction model}: \text{TTHM}\left(\mu\text{gL}^{-1}\right) = 27.5 + 0.3\,\text{Cl} + 2.2\ \text{transphilic} + 3.2\,\text{T} \times \text{transphilic} - 2.3\,\text{Cl}^2$$

The analysis of these models and, especially, the large contribution of interaction effects among the factors confirm that TTHM formation is a complex process. This formation depends globally not only on the type of DOM fraction and its concentration, but as well as on the individual chlorine dose and temperature and on the involved interactions among these three factors. An easier visualization of the combined effects of the three factors can be observed in Fig. 4. The most

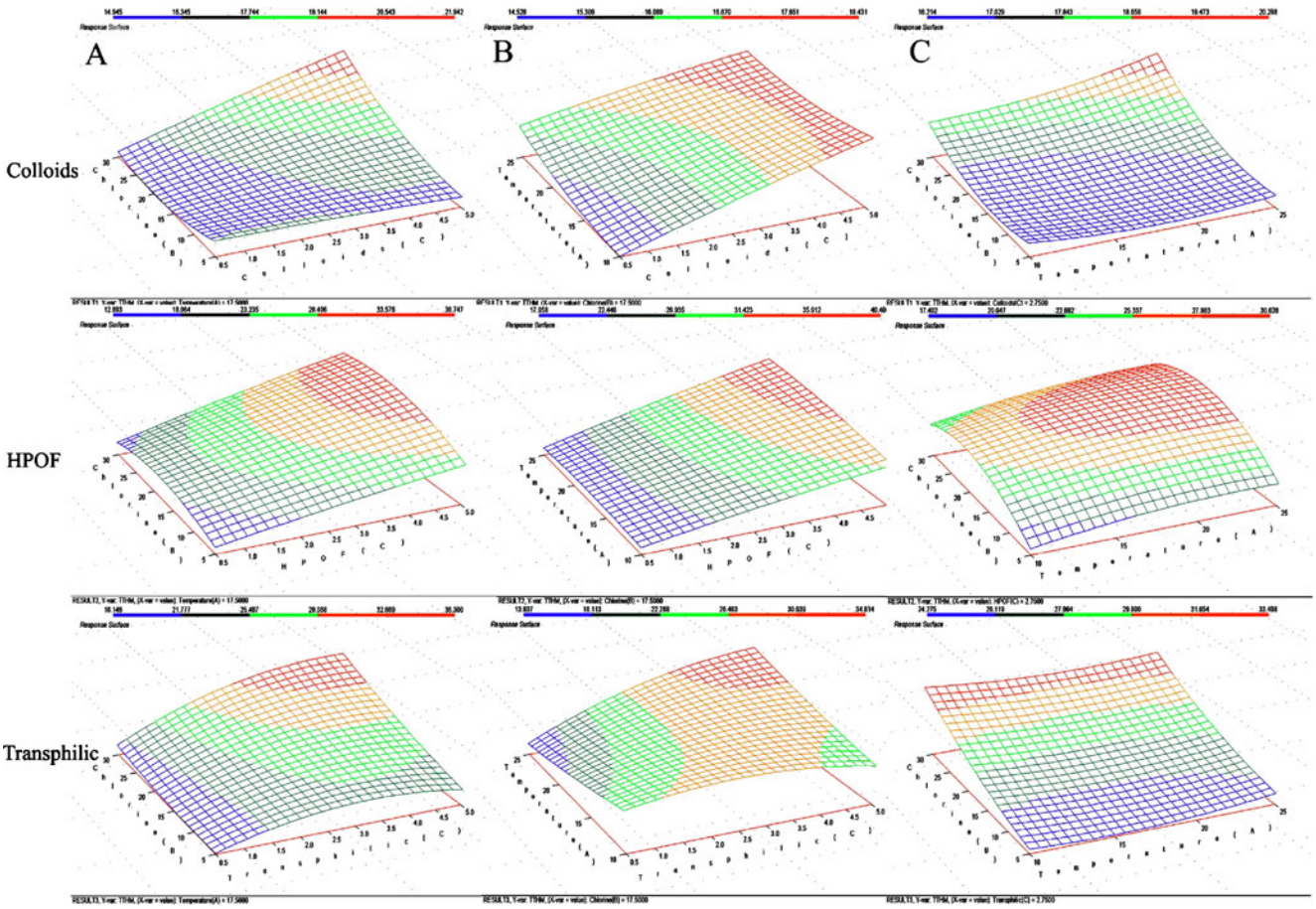

Fig. 4 Response surface of TTHM formation as a function of the three factors and DOM fraction type: **a** DOM fraction vs. chlorine; **b** DOM fraction vs. temperature; **c** chlorine vs. temperature

important is DOM concentration. It positively affects the formation of TTHM, solely or when interacted with chlorine dose and temperature. Although less important, temperature and chlorine also affect TTHM formation.

4 Discussion

Factorial analysis of the water disinfection process is a useful approach for a more comprehensive understanding. This work illustrates a particular study of a local disinfection process in Portugal that leads to the formation of THMs, but it can be extended to other real water works plant management systems. Moreover, it can incorporate the investigation of various factors and their interaction. Combined use of fast screening Placket-Burman and of detailed assessed Box-Behnken experimental designs allowed optimal inferences about more influential parameters in the formation of trihalomethans and of their effects and interactions. Whereas the fast screening did not allow for the detection of the effects of the parameters on bromoform formation probably because of the small number of samples used, the use of the more detailed Box-Behnken design showed clearly this dependence. Therefore, the combined use of both approaches provided a better assessment and reliability of the finally obtained results.

Valuable information was obtained regarding the effect of the DOM fraction type and concentration. The colloidal fraction, richest in nitrogen atoms and poorest in carbon atoms, is approximately responsible for 20–30% of the formation of each individual THM. On average, it contributes a quarter of the formation of the total sum of THMs. A possible explanation for this low contribution in THM production is that this colloidal fraction is more responsible for the production of the other DBPs such as haloacetonitriles and others including N-atoms in their molecules (Ueno et al. 1996).

The fraction where the formation of brominated THMs is more important is the hydrophobic fraction, which is the most carbon enriched fraction. $CHCl_3$ formation strongly depends on the disinfection of the transphilic fraction. Both fractions take similar percentages in the total sum of THM formation—about 40%.

Special attention must be paid when bromide anions are present in raw water. This study reveals that even when only a small quantity of bromide anions exists in the water, the formation of brominated trihalomethanes is highly favored no matter what DOM fraction has been oxidized. This is a consequence of the rapid oxidation of bromide to bromine (hypobromous acid and hypobromite ion). Once formed, bromine is capable of participating in reactions analogous to those of chlorine. The presence of both halogens leads to competition for substitution at suitable carbon atoms in the DOM. Hypobromous acid is a more powerful halogenating agent than hypochlorous acid and this result in a greater incorporation of bromine into DOM. This result is very relevant for risk assessment management since brominated trihalomethanes are considered stronger carcinogen agents than chloroform (Muellner et al. 2007).

5 Conclusions

In order to reduce the concentration of THMs in drinking water, DOM concentrations should be reduced in the water prior to the disinfection. However, taking into consideration the natural complexity of DOM, different operations have to be used to quantitatively remove DOM from the raw water. In fact, the information resulting from this work is in agreement with our previous knowledge about water disinfection. DOM fraction concentration is the most important factor among the investigated ones. No matter which DOM fraction was used, a higher concentration leads to the production of higher amounts of all THMs. Furthermore, water disinfection efforts should focus on the elimination of higher concentrations of each DOM fraction prior to the chlorination. Since the transphilic and HPOF fractions generated 75% of TTHM formed, should the effort to remove the DOM focus on these two organic fractions. The coagulation/flocculation and the filtration stages are the main mechanisms, in a classic water plant treatment, to remove DOM in particular the colloidal and the hydrophobic fraction with a removal of about 70%. The efficiency of the alum treatment for the fractions more hydrophilic is only about 16% (Kim and Yub 2005; Bose and Reckhow 2007). The minimization of the DOM in public water depends, mainly, of a good control of the alum coagulant quantity and raw water pH value.

Special attention must be also paid to the chlorine dose used in disinfection processes. Formation of all THMs is favored by high amounts of chlorine. However, its use is undoubtedly important for the oxidation of raw water and for the disinfection and future avoidance of pathogen regrowth in the distribution system. Chlorine levels should be reduced as low as possible without compromising the microbiological quality of the supplied drinking water, which is the primary concern in the delivery of safe drinking water. In real water works plant management, an investigation of chlorine-DOM fraction type interactions should be undertaken. Temperature appeared also to be significant in THM formation, especially when the DOM concentration and chlorine dose were controlled and constant. Actually, it increases the speed of THM forma-

tion; but the response surface plots reveal that temperature is less significant when chlorine dose and DOM fraction concentrations are low. However, it has been established that temperature decreases the water solubility of THMs and in warm conditions water aeration after chlorination reduces the total amount of THMs present in water.

6 Recommendations and perspectives

The methodology used in this paper is appropriate and can be used in the analysis of other groups of DBPs, mainly the emerging DBPs, like, for example the haloacetic acids, haloacetonitriles, haloketones, or haloacids, which some of them have a more toxic and harmful effect in human health.

The THM reduction in consumption water can be achieved reducing the DOM concentration (mainly the hydrophobic and transphilic fraction) and chlorine dose without compromise the water microbiology quality. Bromide ion concentration control is also very important to minimize the brominated THM formation. These can be previously minimized if the water source contains low concentration of organic and inorganic matter. The use of granular activated carbon and membrane filtration prior the pre-oxidation/disinfection can reduce DOM and consequently the DBPs formation. Moreover, none of the currently available treatment approaches can completely remove pathogens and the precursors to DBP formation. At this moment the solution to minimize the problem is to get a good control in all the process and operational parameters of water treatment.

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