Sensory evaluation of the odors produced during bromophenol formation using a multi-level statistical model


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Abstract
In response to reports of medicinal taste and odor problems in suburban Paris, a lab scale study was conducted to investigate the contribution of different water quality parameters – pH, phenol, bromide, chlorine, temperature and dissolved oxygen levels – on bromophenol medicinal odor formation using the Flavor Profile Analysis (FPA) method. A study of six parameters at 2 levels (64 experiments) analyzed by the FPA method suggests that chlorine at high concentration is more important as a controlling agent than phenol under similar conditions and the ratio of HOBr:Phenol and the time for reaction will control subsequent brominated products of reaction. Results from a three-level statistical model indicate that high pH was associated with lower odor intensities, whereas high levels of chlorine, phenol and temperature were associated with high odor intensities. Potential worst case scenarios of water quality conditions were determined for evaluation by chemical identification and kinetics.

Keywords Bromophenols; chlorination; flavor profile analysis; medicinal odor; phenols

Introduction
In the Le Pecq service area of the Paris suburbs medicinal taste and odor problems have been observed. The suggested cause of this problem is the slow formation of brominated phenols that may occur upon chlorination of phenol contamination in a ground water that contains bromide. This hypothesis has not been verified by chemical analysis because of the low (ng/L) odor threshold concentrations (OTCs) of brominated phenols and the transient nature of the problem. The chlorination of phenols has been rigorously studied at mg/L concentrations of phenol and chlorine in distilled water (Lee, 1967). Phenol chlorination is known to be a successive addition process, and when sufficient chlorine is added, the benzene ring will be broken and the chlorophenol destroyed. This has never been studied at water treatment levels of µg/L concentrations of phenols and mg/L concentrations of chlorine alone or in the presence of bromide. Figure 1 presents the expected reaction sequence for the bromination of phenols after HOBr forms from the reaction of HOCl with bromide ion. The formation of bromophenols is also a function of pH. The optimum values for development of bromophenols occurs between pH 8–9, as studied by Ventura and Rivera (1986).

The bromophenols have the same type of odors as chlorophenols (iodoform, phenolic, medicinal), (Lee, 1967) however the OTC of some brominated phenols are in the ng/L range (Whitfield et al., 1988; see Figure 1). The OTCs of bromophenols follows approximately the same pattern as the chlorophenols. The 2-bromophenol and 2,6-bromophenol have lowest OTCs of 30 ng/L and 0.5 ng/L, respectively (Whitfield et al., 1988). These OTCs are near the lowest OTCs observed for drinking water chemicals, rivaling even the earthy/musty odorous, Geosmin and Methyl Isoborneol (MIB).
This project was designed to study how the medicinal taste and odor caused by bromophenols are observed in drinking water after final chlorination in the presence of µg/L concentrations of phenols and bromide. We also wish to develop a set of potential worst case scenarios to choose experimental conditions for pipe loop experiments and/or lab chemical kinetic studies.

**Methods**
Flavor profile analysis was used for the evaluation of the odor character, the strength of the odor formed and the time after mixing that the odor formed (APHA Standard Methods, 1995). Application of the FPA sensory technique for tastes-and-odor problems in drinking water are given elsewhere (e.g. Suffet *et al.*, 1995). Evian bottled water was used for sample preparation. All samples were stored in the dark in amber glass containers and left unstirred. The FPA panelists independently studied each sample’s odor, and used the quantitative FPA, 7 point scale, to note any odor characteristics and their intensities. The quantitative FPA, 7 point scale consists of the symbol )( [threshold or 1, 2, 4, 6, 8, 10, 12. The experimental parameters used are listed in Table 1.

**Statistical analysis**
In order to obtain the maximum power from the factorial design, a multilevel analysis of a continuous response was completed. This methodology takes into account potential biases introduced by panelists at the different time points. For example, if a panelist consistently overestimates or underestimates the intensity of an odor as compared to the rest of the panel, the method compensates for this bias. In addition, if a panelist performance improves or decreases over time, the method will also compensate for it. The data was analyzed using a model with three levels, in which each data point $Y_{ijk}$ corresponds to experimental unit $i$, evaluated at time point $j$ by panelist $k$. Day 2 was chosen as the baseline.

**Figure 1** Reaction scheme for the formation of bromophenols (Flavor threshold concentration from Whitfield *et al.*, 1988). Note: At each arrow an HOBr molecule is reacting.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.5 and 8.5</td>
</tr>
<tr>
<td>Phenol level</td>
<td>0.2 and 1.0 µg/L</td>
</tr>
<tr>
<td>Bromide level</td>
<td>Evian and about the Le Pecq conc. 20 and 100 µg/L</td>
</tr>
<tr>
<td>Chlorine level</td>
<td>0.1 and 0.3 mg/L</td>
</tr>
<tr>
<td>Temperature</td>
<td>10 and 20 degrees C</td>
</tr>
<tr>
<td>Dissolved oxygen level (DO)</td>
<td>0 and 8 ppm</td>
</tr>
<tr>
<td>Analyses time</td>
<td>2, 4, 24 and 48 hours (day 0, 1 and 2)</td>
</tr>
</tbody>
</table>

Table 1 Specific values and units for each experimental factor level and time point.

Water matrix: Evian bottled water. Water samples were heated to 45°C for FPA for 30–60 min.
\[
Y_{ijk} = \beta_{0jk} + \sum_{h=1}^{6} \beta_{hij} \cdot X_{hij} + \sum_{f=1}^{6} \sum_{g=f+1}^{6} \beta_{fgij} \cdot X_{fij} \cdot X_{gij} + \varepsilon_{ijk}
\]  

(1)

\[
\beta_{0jk} = \gamma_{02k} + U_{00k} \cdot I(t_j = 0) + U_{01k} \cdot I(t_j = 1) + \gamma_{00} \cdot I(t_j = 0) + \gamma_{01} \cdot I(t_j = 1)
\]  

(2)

\[
\gamma_{02k} = \gamma_{02} + U_{02k}
\]  

(3)

Model Assumptions:

\[
U_{0jk} \sim N(0, \pi^2_j)
\]

\[
\text{Cor}(U_{0jk}, U_{0j'k}) = \rho_{j,j'}
\]

\[
\varepsilon_{ijk} \sim N(0, \sigma^2)
\]

In formula 1, \(\beta_{hij}\) represents the coefficient for the fixed effect of factor \(h\) at time \(j\). \(\beta_{fgij}\) represents the coefficient for the interaction of factor \(f\) with factor \(g\) at time \(j\) – only combinations of two experimental factor interactions with time were considered estimable in the dataset due to the lack of replicates; all higher order interactions are assumed to equal zero. \(\beta_{0jk}\) is described by formula 2; \(\beta_{0jk}\) represents the combination of the effect of panelist \(k\) at time \(j\) \((U_{0jk})\) with the effect of having all experimental factors set to the low level at time \(j\) \((\gamma_{0j})\). These effects are represented as additive terms to the day 2 baseline \(\gamma_{02k}\), governed by indicator functions of the form . Formula 3 indicates that the baseline is composed by both the panelist effect and the effect of having all experimental factors set to the low level at day 2. The most evident limitation of this model is that it assumes that the error terms are normally distributed around a mean of zero with a fixed variance. However, the process that generated the data limited the outcome to a scale of seven possible values, and only six of those are present in the dataset, therefore a multinomial distribution for the errors could be more suitable.

**Fixed effects.** Selection of the fixed effects to incorporate into the model followed an ad-hoc backward elimination criterion. This set of fixed effects consists of Time interacting with Temp, pH*HOCl, pH*phenol, pH*DO and the corresponding main effects. Bromide was deemed to not cause any significant effect; therefore the low level concentration of 20 µg/L was sufficient for the reactions to occur. After the final selection of fixed effects was performed, the data was fitted to the model using SAS PROC MIXED with the Restricted Maximum Likelihood option (Singer, 2003). The estimated coefficients that are significant are listed in Table 2 for pH, phenol, HOCl and temperature. For example, pH has a significant effect on day 0 and 1 only; higher pH decreases the average intensity by 2 units. The statistical model found that bromide concentration was not statistically significant, therefore the low level concentration was sufficient to produce bromophenols.

**Random effects.** The random effects of the panelists at each time point are assumed to be distributed normally around a mean of zero, however these effects are allowed to have their

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day</th>
<th>Change of odor intensity</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0</td>
<td>-1.87</td>
<td>0.0053</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.006</td>
<td>0.5162</td>
</tr>
<tr>
<td>pH</td>
<td>1</td>
<td>-1.97</td>
<td>0.0036</td>
</tr>
<tr>
<td>phenol</td>
<td>1</td>
<td>1.78</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.04</td>
<td>0.2834</td>
</tr>
<tr>
<td>Temp</td>
<td>2</td>
<td>0.91</td>
<td>0.0004</td>
</tr>
<tr>
<td>HOCl</td>
<td>2</td>
<td>1.59</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Br</td>
<td>2</td>
<td>0.04</td>
<td>0.3162</td>
</tr>
</tbody>
</table>
own variance at each time point, reflecting the fact that the panelists might become better attuned to the odors they are experiencing or, conversely, might become less sensitive to such odors. The covariance among the panelist’s effects across time points was left unstructured. Model fit diagnostics were assessed by verifying the normality assumption of the residuals and the assumption of constant variance for the residual distribution.

An important limitation of this model is that the maximum intensity estimate of the FPA results is 5.7, whereas the maximum observed response is 10. Thus, the model tends to attenuate the effect of high responses, perhaps due to invalidity of the assumption for certain high-order interactions to equal zero. Further examination of the residuals of the fit, without incorporating the random effects per panelist, across all three time points were conducted to verify the validity of the assumptions made about the random effects (Snijders and Bosker, 2002). In addition, those plots were also used to monitor the performance of each panelist (data not shown).

Results and discussion

Figure 1 shows the reaction scheme for the formation of bromophenols. The flavor threshold concentration and odor type of the bromophenols in water are included from Whitfield et al. (1988). The amount of 2-bromophenol versus 4-bromophenol that is formed controls initial odor formation. However, as a second bromine is added to the benzene ring, the odor can increase 2- to 2,6-dibromophenol or increase from 4- to 2,4-dibromophenol. Which pathway is followed and how much of each pathway is followed under any one condition is not understood at µg to ng/L concentrations.

Reproducibility of Flavor Profile Analysis (FPA) experiments

A comparison of the error between multiple analyses of five different runs with the same experimental conditions conducted during the study is shown in Figure 2. The sample parameters in Figure 2 appear to be a very reactive condition as the trend usually shows high increases at 24 hrs and then decreases in 48 hrs. Maximum intensity values were at 24 hrs, and the maximum intensity value observed for any of the individual runs was 5.0. The standard deviation (SD) among the five runs at 24 hrs was ± 1.8 FPA intensity units. The SD at 48 hrs was within ± 1.2 FPA intensity units. The results of the statistical model in Figure 2 show two times the standard error, ± 0.8 FPA intensity units at both 24 and 48 hrs. These results indicate that the model followed the same trends as the observed results. Therefore the model can be used to represent the data set for quantitative comparison of experimental [Image of Figure 2]
conditions across time points. The results also show that the data was sufficient to make a judgment of the worst case conditions for the pipe loop experiment, and this run represents a high FPA intensity. This is a condition to consider for subsequent worst case kinetic studies.

The large difference between intensity values (± 1.8 FPA Intensity units) for the five runs at 24 hrs may be due to the dynamics of the reaction kinetics, as these samples were continuously reacting and changing with time. It is important to note that slight differences of solution concentration for a particular kinetic run may produce this large error. These samples were run at 20°C without shaking and then heated to 45°C for 1/2 to 1 hour for evaluation by FPA. Shaking and increasing the temperature for the FPA analysis also can affect the reaction kinetics of the sample. The time for heating from 20 to 45°C for FPA evaluation was maintained at a specific heating time of 30 minutes or less.

Another concern was that chlorine odor dissipation at near threshold and medicinal odor (phenol, iodoform odor) occurrence were difficult to separate by the FPA panel. The odors appear to possess similar characteristics especially at low levels. Thus, in order to simplify the FPA data all medicinal and chlorine odors that were perceived by the panelists were treated as medicinal odors. This was done as the chlorine OTC is about 0.3 mg/L (Krasner and Barrett, 1984) and this or 0.1 mg/L chlorine were the concentrations used in the experiment. The FPA panelists had not been intensively trained to develop a consistent scale for FPA intensity evaluation. Therefore the trend of the data is most important. The statistical approach presented adjusted the individual panelist intensity scale for a more consistent overall FPA intensity scale for total panel results. Figure 3 shows the odor intensity level remains with a high standard error of 1–2 units even after the individual panel intensities are adjusted.

**Evaluation of estimated data trends**

High concentration conditions of chlorine, phenol, and DO, combined with higher temperature clearly show a stronger reaction rate reaching higher intensities within 48 hours (for DO, chlorine and high temperature), as seen in Figure 3. The run with high chlorine and phenol achieves its maximum intensity within 24 hours, whereas a separate run of these two factors in high concentration plus higher temperature shows a trend of increasing intensities over time, assuming that only bromophenols are being formed. The run with both chlorine and phenol in high concentrations reaches a maximum FPA intensity of 4 ± 0.8 by 24 hours. This indicates that both chlorine and phenol are controlling factors in these reaction kinetics. Thus, low concentration of bromide is sufficient to produce bromophenols. This is confirmed by the stoichiometry of the reactions:

\[ \text{HOCl} + \text{Br}^- \rightarrow \text{HOBr} + \text{Cl}^- \]  

(1)

Under high chlorine conditions of 0.3 mg/L as Cl, we have excess chlorine compared to bromide and therefore there is a limited stoichiometric amount of HOBr formed. Thus, the maximum HOBr that is formed is between 20 and 100 µg/L as Br.

\[ \text{HOBr} + \text{C}_6\text{H}_5\text{OH (Phenol)} \rightarrow \text{C}_6\text{H}_4\text{OH(Br)} + \text{H}_2\text{O} \]  

(2)

Under the conditions of 0.2 to 1 µg/L of phenol, the experimental conditions are phenol limiting. The excess HOBr present should inhibit the formation of chlorophenols from the excess chlorine present. This is because HOBr reacts 100 times faster with phenol than HOCl (Echigo and Minear, 2001). This indicates that only bromophenols should be forming in the system.
Initial evaluation of FPA data for the highest bromophenol formation

The experimental conditions could produce successive bromophenols of different odor threshold concentration. Individual or more likely mixtures of bromophenols will sometimes be present. The FPA evaluations were completed over time to follow the overall odor that is present at any time. The following evaluation is based upon odor formation only at two pHs. Echigo and Minear (2001) presented the effect of pH on the second order rate constant for bromination of phenols. Between pH 6.5 and 8.5, the rate constant increased from about $1 \times 10^4$ to about $3 \times 10^5$ which is 30 times quicker. Therefore, the phenolate ion controls the reaction rate.

Experiments at pH 8.5

Table 3 summarizes the highest FPA intensities observed for all experiments at high pH (8.5) at dissolved oxygen (DO) levels of 0 and 8 ppm. Literature reports that at this pH, kinetic experiments are at least thirty times quicker in reaction time than at lower pHs. However, the reaction stoichiometry (HOBr:Phenol ratios) in the literature was much more favorable than tested here and reactants were at much higher concentration levels. The phenolate ion which is present at higher concentrations at higher pH is considered the most reactive phenol species to react with HOBr (Echigo and Minear, 2001).

Figure 2 and Table 3 together show the 2 most odorous runs at pH = 8.5 and DO levels of 0 and 8 ppm are for runs, R18–S2 and R7–S5. The highest FPA intensities are observed in R7–S5 at or after 24 hours with all high concentration reactants. The run, R18–S2 does not correspond to the run R7–S5, and the statistical analysis from Figure 2 of the high levels
produced the highest odor intensity as low temperature, low phenol and low chlorine are used. However, the FPA intensity occurs slowly at \( t = 48 \) hrs. These runs indicate the probable formation of the most odorous, 2,6-dibromophenol at pH 8.5 occurs near 24 hours. Both these runs would make good candidates to be run in a pipe loop experiment, and to evaluate them by simultaneous chemical and FPA analysis.

**Experiments at pH 6.5**

Table 3 also summarizes the highest FPA intensities observed for all experiments at low pH (6.5) at DO levels of 0 and 8 ppm. Literature reports that at this pH kinetic experiments have a reaction rate at least 30x slower than at higher pHs (Echigo and Minear, 2001). However, the reaction stoichiometry (HOB:Phenol ratios) in the literature was much more favorable than tested here and higher concentration levels were studied.

The low pH runs showed high odor intensities in 24 hours. Table 3 shows the statistical analysis indicated that high levels were generally observed with combinations of high levels of HOCl, phenol, and temperature. Yet run 11 sample 2 at low pH had a high odor intensity value of 6 at 24 hours despite having lower levels of HOCl, phenol, and temperature. This runs seems to not fit the statistical analysis, however there are several possible explanations for this deviation from the statistical model. The maximum fitted value for intensity from the statistical model was 5.7, whereas the maximum observed response was 10, and there was a wide range of fitted values for each observed response, especially for lower intensities. This attenuation, the large differences in intensity observed in running samples under the same conditions, such as the ± 1.8 FPA intensity unit spread observed in Figure 2 at 24 hours, and other limitations of the data set forcing the assumption that there was no experimental variation introduced by preparation of the experimental parameters could help account for the deviations observed in this run as in the run R18–S2 at high pH. Additionally, while the model is sufficient to make qualitative assessments about the different experimental factors affecting odor formation, its predictive power is unknown and needs to be tested by chemical evaluation in the future.

Table 3 outlines the 6 most odorous runs at pH 6.5 and DO levels of 0 and 8 mg/L. R6–S1 is a run for comparison only. At DO = 8 mg/L the R2–S8 and the R2–S5 runs do not have the highest chlorine and bromide:phenol ratio, however they were runs with the high FPA intensities. The difference between R2–S8 and R2–S5 is that the temperature is lower for R2–S5 and in this run a lower initial FPA intensity at the beginning of the experiment is observed. The run R11–S2 has 3 parameters less than maximum (temperature, phenol and chlorine concentrations) but a higher bromide concentration than R2–S5 and R2–S8. Only one high intensity was noted on run R11–S2, and thus it does not make a good candidate for the pipe loop experiments. The run R2–S8 should be run in the pipe loop experiments. All pipe loop experiments should be analyzed simultaneously by chemical and FPA analyses.

Table 3 also shows that at DO = 0 mg/L the maximum concentrations of all parameters,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low pH</th>
<th>High pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2–S8</td>
<td>R11–S2</td>
</tr>
<tr>
<td>Dissolved O(_2), (mg/L)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Bromide, (µg/L)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Phenol, (µg/L)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Chlorine, (mg/L)</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Time to Maximum, (hrs)</td>
<td>( &gt;48 )</td>
<td>24</td>
</tr>
<tr>
<td>Maximum FPA Intensity</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

* R6–S1 is a run for comparison at pH 6.5 and DO = 0
as in R6–S1, do not show the highest FPA intensity. However, the combination of the highest parameters at the lower temperature (R13–S3) does reach a higher FPA intensity and follows the trend of run R6–S1. The run, R13–S2 shows an increasing FPA intensity, which dissipates slowly after 24 hours but does not reach the high FPA intensity levels observed in run R18–S5. The run R18–S5 shows the highest FPA response at 24 hours and then a little less response at 48 hours. This run is at the highest concentration levels except for phenol, and thus has the highest chlorine and bromide:phenol ratio, and would be a good candidate to be run in the pipe loop experiment and to repeat simultaneously by chemical and FPA analysis, as it forms odors at the highest FPA intensity.

**Conclusions**

The statistical model allowed an efficient comparison of experimental conditions. The ratio of HOBr:Phenol and the time for reaction will control whether subsequent brominated products of reaction can occur. While it still remains to be seen which conditions have resulted in the taste and odor problems in France, there are several likely candidates based upon the FPA experimental screening experiment. It is recommended that the following conditions be tested using the pipe loop experiments for further confirmation and with simultaneous chemical and FPA analytical studies. From Table 3 the suggested candidate runs with the highest odor forming potential are runs: R7–S5 (DO = 8) and R18–S2 (no DO) at pH 8.5 and R2–S8 (DO = 8) and R18–S5 (no DO) at pH 6.5. With the exception of R18–S2 these runs concur with the trend of high levels of chlorine, phenol and temperature being associated with high odor intensities indicated by statistical analysis.

**References**


