Influence and Interference by Thymol added as a Biocide on the Analysis of Precipitation Samples

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1. INTRODUCTION

Acid rain has attracted attention as a regional-scale environmental issue in Europe and North America since the beginning of the 1970's. Recently, the acid rain issue has extended to East Asia because of a significant increase in anthropogenic emissions of sulfur dioxide, nitrogen oxides, and other substances to the atmosphere due to high economic growth in the region. The region is increasingly facing threats of acid deposition, and it is becoming essential to struggle against these threats through regional cooperation and the efforts of individual countries. The Acid Deposition Monitoring Network in East Asia (EANET) was organized in 2000 to create a common understanding among countries and organizations of the status of acid deposition in the region. Regular phase monitoring started in 2001 based on Technical Documents in East Asia1).

Precipitation samples should be collected without degeneration. Thymol is a biocide effectively used to prevent samples from degenerating2, 3). Technical Documents recommends using thymol as a biocide unless refrigeration can be used for the preservation of precipitation samples. However, the addition of thymol to precipitation samples interferes with the determination of chemical species in some analytical methods.

This paper evaluates the influence and the interference by thymol on pH analysis and colorimetric methods, especially indophenol blue method for NH₄⁺ analysis of analyzing precipitation samples in the actual use of thymol as a biocide. The ion chromatograph has been used worldwide for the determination of chemical species. However colorimetric methods or titration methods have been used for the determination of chemical species in some countries in the above EANET.

2. EXPERIMENTAL

2.1 Solubility of Thymol for Deionized Water and Dilute KCl Solution

The solubility of thymol for deionized water and dilute KCl solution was examined. Ten, 20, 40, 100, 200, and 500 mg thymol were added to 100 ml deionized water and dilute KCl solution (10⁻⁴ M). The reason that dilute KCl solution was used is described in 2.2. The solutions were left at room temperature (approximately 20°C) for 1 day. After 1 day, the thymol that did not dissolve was filtrated and weighted. The solubility was calculated by the difference of thymol between the added and remaining amounts.

2.2 Influence on pH Measurement for Dilute KCl Solution

Deionized water is not suitable for the examination of pH measurement because it is easily influenced by carbon dioxide in the atmosphere. A dilute (10⁻⁴ M) of potassium chloride (KCl) solution was, therefore, used as a neutral solution to examine the influence of thymol on pH measurement. The pH value was measured with 0, 10, 40, 200, and 1000 mg thymol added to 100 ml dilute KCl solution. The pH value of the dilute KCl solution was measured 1 hour after the thymol was added.

2.3 Influence on pH Measurement for Precipitation Samples

2.3.1 Collection of precipitation samples

Four identical filtering-type bulk samplers4) were used to collect precipitation samples. The collection was conducted on the roof of the institute (25 m above ground level) in summer (average air temperature: about 24°C; maximum air temperature: about 40°C). Fifty mg, 200 mg, 1000 mg, or 5000 mg thymol was added to the four samplers before the collection was started. The samples were collected weekly and stored in polyethylene bottles at room temperature (about 15°C). The weekly precipitation amount in Japanese fiscal year (April–March) 2000 is
as follows: mean 436.5 ml (19.2 mm), maximum 2750 ml (121.2 mm), minimum 0 ml (0 mm) and standard deviation 614.9 ml (27.1 mm).

2.3.2 pH measurement of precipitation samples

After collection, the samples were analyzed four times: as soon as possible after collection, 1 month later, 2 months later, and 3 months later.

2.4 Influence on Colorimetric Methods

The influence on colorimetric methods for NH$_4^+$ (indophenol blue method)\(^5\), Cl$^-$ (Hg (SCN)$_2$ method)\(^6\), NO$_3^-$ (HOC$_6$H$_4$000Na method)\(^7\), and SO$_4^{2-}$ (barium sulfate turbidimetry)\(^8\) was examined. Seven series of 100 ml NH$_4^+$ standard solution (about 0.1 nM) with different amounts of thymol (0, 1, 2, 3, 4, 5, and 20 mg) were analyzed by the indophenol blue method. Five series of 100 ml Cl$^-$ (about 0.7 nM), NO$_3^-$ (about 0.2 nM), and SO$_4^{2-}$ (about 1.0 nM) standard solution with different amounts of thymol (0, 10, 40, 200, and 1000 mg) were analyzed by the Hg (SCN)$_2$ method, the HOC$_6$H$_4$000Na method, and barium sulfate turbidimetry, respectively.

3. RESULTS AND DISCUSSION

3.1 Solubility of Thymol for Deionized Water and Dilute KCl Solution

The dissolved amounts of thymol were 9.0, 18.8, 32.4, 41.9, 66.3, and 92.2 mg for 10, 20, 40, 100, 200, and 500 mg of thymol added to 100 ml deionized water, respectively. Similarly, the dissolved amounts for the dilute KCl solution were 8.9, 15.3, 27.0, 44.8, 63.9, and 99.4 mg. In Technical Documents, 400 mg thymol per 1000 ml solution is recommended\(^1\). The result shows, however, a possibility of excessive dissolution over the recommendation of Technical Documents. A disadvantage of excessive dissolution over the recommendation is described in the following section.

3.2 Influence on pH Measurement for Dilute KCl Solution

The dependence of the pH value of the dilute KCl solution on the amount of thymol added is shown in Fig. 1. The pH value decreased as the amount of added thymol was increased. This result was probably due to the dissociation of thymol, as shown in Fig. 2. In Technical Documents, 400 mg thymol per 1000 ml solution is recommended as mentioned above\(^1\). Fig. 1 shows that the pH value was affected only a little by the amount of thymol added, up to 400 mg/1000 ml dilute KCl solution. However, with the use of thymol for precipitation samples, the limitation that will be discussed in 3.3.2 was accompanied.

3.3 Influence on pH Measurement for Precipitation Samples

3.3.1 Collection of precipitation samples

Twelve series of samples (No. 1 ~ No. 12) were collected. The precipitation levels are given in Table. No significant (p > 0.05) difference on precipitation was caused by the difference in the amount of thymol added before starting collection, showing that the four samples within each series could be evaluated equally.
Table  Precipitation levels from No.1 to No.12

<table>
<thead>
<tr>
<th>Amount of thymol added</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
<th>No.5</th>
<th>No.6</th>
<th>No.7</th>
<th>No.8</th>
<th>No.9</th>
<th>No.10</th>
<th>No.11</th>
<th>No.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>50mg</td>
<td>556</td>
<td>1066</td>
<td>490</td>
<td>279</td>
<td>541</td>
<td>197</td>
<td>570</td>
<td>512</td>
<td>295</td>
<td>267</td>
<td>619</td>
<td>204</td>
</tr>
<tr>
<td>200mg</td>
<td>556</td>
<td>965</td>
<td>432</td>
<td>278</td>
<td>526</td>
<td>187</td>
<td>562</td>
<td>510</td>
<td>301</td>
<td>281</td>
<td>611</td>
<td>216</td>
</tr>
<tr>
<td>1000mg</td>
<td>583</td>
<td>1071</td>
<td>480</td>
<td>276</td>
<td>523</td>
<td>190</td>
<td>564</td>
<td>511</td>
<td>292</td>
<td>269</td>
<td>596</td>
<td>212</td>
</tr>
<tr>
<td>5000mg</td>
<td>578</td>
<td>1068</td>
<td>506</td>
<td>278</td>
<td>550</td>
<td>192</td>
<td>589</td>
<td>528</td>
<td>325</td>
<td>283</td>
<td>660</td>
<td>220</td>
</tr>
</tbody>
</table>

Fig. 3 Plots on the amount of thymol vs. pH.

ΔpH is defined as pH_{ijk} - pH_{ij} (0 month) for i = amount of thymol added before starting collection (50, 200, 1000, and 5000 mg), j = the series number of the precipitation samples (No. 1 ~ No. 12), and k = the number of months before measurement (1 month, 2 months, and 3 months).

3.3.2 pH measurement for precipitation samples

The plots of the amount of thymol vs. ΔpH are shown in Fig. 3, where ΔpH is defined as pH_{ijk} - pH_{ij} (0 month) for i = amount of thymol added before starting collection (50, 200, 1000, and 5000 mg), j = the series number of the precipitation samples (No. 1 ~ No. 12), and k = the number of months before measurement (1 month, 2 months, and 3 months). The horizontal axis is shown by the unit of mg/100 mL sample, which was calculated based on the amount of thymol (50, 200, 1000, and 5000 mg) / the precipitation collected, as shown in Table. The pH values in some samples increased when the amount of thymol contained in the samples was less than 10 mg/100 mL sample. This result was probably due to an inadequate amount of thymol. In contrast, the pH values decreased slightly when the amount of thymol contained in the
samples was more than 100 mg/100 ml sample. Technical Documents requires that thymol should be added before starting collection to prevent samples from degenerating during the collection. However, it is impossible to predict the amount of precipitation that will be collected. As a result, the amount of thymol that will be contained in the precipitation samples will be different for each sample. A limitation is presented here. The amount of thymol contained in the samples will be small when the amount of precipitation is large. A small amount of thymol will result in an increase in the pH value, as shown above. On the other hand, an excess amount of added thymol will lead to a decrease in the pH value.

3.4 Influence on Colorimetric Methods

3.4.1 Influence on indophenol blue method

Fig. 4 shows the change in absorption in the indophenol blue method when different amounts of thymol (0, 1, 2, 3, 4, 5, and 20 mg) were added to the seven series of NH₄⁺ standard solution. The absorption decreased with an increase of the amount of thymol added.

A chemical reaction occurred in the indophenol blue method, as shown in Fig. 5 (a). The chemical structures of phenol and thymol are also shown in Fig. 5(b). The blue color in the indophenol blue method is due to the product caused by the reaction shown in Fig. 5 (a). The chemical structure of thymol, which is similar to that of phenol, makes it possible to substitute thymol for phenol through a competitive reaction, which is responsible for the decrease in the formation of the product that is due to the blue color. As a result, the absorption in the indophenol blue method decreases with

![Fig. 4](image-url)  
**Fig. 4** Change in absorption in the indophenol blue method by the addition of thymol

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\begin{align*}
\text{NH}_3 + \text{NaOCl} & \rightarrow \text{NH}_2\text{Cl} + \text{NaOH} \\
\text{NH}_2\text{Cl} + \text{HO} - \rightarrow 2\text{NaCl} - 2\text{H}_2\text{O} \\
\text{HO} - \rightarrow \text{Cl}^-\text{N} = \text{Cl}^-\text{O} + \text{HCl}
\end{align*}
\]

(a)

![Fig. 5](image-url)  
**Fig. 5** (a) Chemical reaction occurred in the indophenol blue method, and (b) chemical structure of phenol and thymol
the addition of thymol.

The decrease in the absorption is dependent on the amount of thymol added to the sample solution. However, it is impossible to control the amount of thymol to be contained in sample solution, as mentioned above. Consequently, the indophenol blue method cannot be recommended for samples to which thymol is added before starting collection.

3.4.2 Influence on other colorimetric methods

The influences on the Hg (SCN)₂ method, the HOC₆H₄COONa method, and barium sulfate turbidimetry are shown in Fig. 6. The addition of thymol apparently had no influence on these colorimetric methods.

4. CONCLUSIONS

The influence and the interference of thymol, which is recommended for the preservation of precipitation samples unless refrigeration can be used, were examined with respect to the analysis of samples. The pH value of dilute KCl solution decreased when the amount of thymol added was more than 100 mg/100 ml KCl solution. In contrast, the pH value increased when the amount of thymol added was less than 10 mg/100 ml precipitation sample. An influence on the analysis of pH by the addition of thymol was inevitable because it was impossible to predict the amount of precipitation collected. The absorbance in the indophenol blue method decreased with the addition of thymol because its chemical structure is similar to that of phenol. Using the indophenol blue method to analyze precipitation samples to which thymol is added before starting collection is not recommended. In contrast, the addition of thymol did not influence other colorimetric methods such as the Hg(SCN)₂ method, the HOC₆H₄COONa method, and barium sulfate turbidimetry.

REFERENCES

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ABSTRACT

Thymol (2-isopropyl-5-methyl phenol) is recommended for the preservation of precipitation samples to suppress the microbial activity and conversion of organic acids unless refrigeration can be used. However, the addition of thymol to precipitation samples influences and interferes with the analysis of samples. The pH value of dilute (10⁻⁴ M) KCl solution decreased when the amount of thymol added was more than 100 mg/100 ml dilute KCl solution. In contrast, the pH value increased when the amount of thymol added was less than 10 mg/100 ml precipitation sample. The absorbance in the colorimetric method (indophenol blue method for NH₄⁺ analysis) was decreased by the addition of thymol. This result was probably caused by the competitive reaction of thymol and phenol through their similar chemical structures. The decrease in the absorbance was dependent on the amount of thymol added. Therefore, the indophenol blue method cannot be recommended for samples to which thymol is added before starting collection because it is impossible to add an appropriate amount of thymol.

Key Words: Acid rain, Indophenol blue method, pH, Precipitation, Thymol