研究論文

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

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殺菌剤として添加されたチモールが降水試料の分析に与える影響・妨害

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要 旨

現在、酸性雨現象は、雨、雪や霧を対象とした湿性沈着と非降水時のガスや粒子を対象とした乾性沈着の両者を含めて評価されているが、測定の基本はいかに正確に降水を捕集し化学成分を分析するかという点から出発する。降水中の化学成分は微生物活動、光や熱の影響、大気中気体成分の吸収ならびに共存する粒子状物質との相互作用によって変質する。そのため、試料採取においては、冷却、ろ過、遮光ならびに密閉などの対策をとる必要がある。このうち、冷却の目的で冷蔵庫内蔵型のサンプラーの使用が推奨されているが、どうしても高価な装置とならざるをえない。一方、酸性雨現象は大陸内程度の中-長距離輸送による酸性物質の大気中ならびに雲中での硫酸や硝酸への化学的変化過程に基づくと考えられるため、広域的な監視網の形成が必要である。この目的で、2000年から日本、中国、韓国を中心とした東アジア酸性雨モニタリングネットワーク (EANET) が立ち上げられ、2001年からは測定が開始されている。この中で、夏季の試料ならびに熱帯地域周辺の国々の試料においては、試料採取後の化学成分の変質防止が大きな課題となっている。チモール (2-イソプロピル-5-メチルフェノール) は降水試料を採取・保存する際に冷蔵庫が使用できない場合の殺菌剤としてその使用が推奨されている。確かにチモールは有効な殺菌剤であることが証明されてきたが、他方、チモールは降水試料を分析する際に分析に影響・妨害を与える可能性がある。本研究では、事前に容器中に一定量のチモールを添加する採取法を想定し、そのことが、降水試料の pH 測定や吸光光度法での化学成分測定におよぼす影響を調べた。

10^{-4} M (mol/l) の KCl 溶液の pH はチモールの添加量が 100 mg/100 ml を超えると低下した。その一方、実際の降水試料のいくつかではチモールの添加量が 10 mg/100 ml 以下であると pH は上昇し、100 mg/100 ml 以上であると pH は低下した。NH₄⁺ (アンモニウムイオン) を分析する際に吸光光度法であるインドフェノール青法を用いる場合はチモールを添加することにより吸光度が低下したが、これはインドフェノール青法を用いる際に添加するフェノールと殺菌剤として添加したチモールの競争反応によると考えられる。インドフェノール青法において吸光度がどの程度低下するかは添加されたチモールの濃度によるが、試料採取する際に添加するチモール量は試料採取容器をセットする前に一定量を加えるため、試料採取後にチモール濃度が試料中でいくらになるかは調整できない。このことから NH₄⁺ を分析する際にインドフェノール青法を用いる場合はチモールを添加することは分析上適さない。

また、同様の吸光光度法である、塩化物イオン分析用のチオシアン酸第二水銀法、硝酸イオン分析用のサリチル酸ナトリウム法および硫酸イオン分析用の硫酸バリウム比濁法についても、吸光度に与えるチモールの影響を調べたが、いずれも影響は認められなかった。

キーワード：酸性雨、インドフェノール青法、pH、降水、チモール

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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 degenerating^{2, 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 NH_4^+ 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^- ($\text{Hg}(\text{SCN})_2$ method)⁶⁾, NO_3^- ($\text{HOC}_6\text{H}_4\text{COONa}$ 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 $\text{Hg}(\text{SCN})_2$ method, the $\text{HOC}_6\text{H}_4\text{COONa}$ 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 1,000 ml solution is recommended¹⁾. 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 above¹⁾. 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.

Fig. 1 Dependence of the pH value of the dilute KCl solution on the amount of thymol added

Fig. 2 Dissociation of thymol

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

Fig. 3 Plots on the amount of thymol vs.

ΔpH. ΔpH is defined as $pH_{ijk} - pH_{ij(0\text{ month})}$ 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 $pH_{ijk} - pH_{ij(0\text{ month})}$ 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 collection¹⁾. 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

Fig. 4 Change in absorption in the indophenol blue method by the addition 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 NH_4^+ 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

(a)

(b)

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 $\text{Hg}(\text{SCN})_2$ method, the $\text{HOC}_6\text{H}_4\text{COONa}$ 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

Fig. 6 Influences on the $\text{Hg}(\text{SCN})_2$ method, the $\text{HOC}_6\text{H}_4\text{COONa}$ method, and barium sulfate turbidimetry

other colorimetric methods such as the $\text{Hg}(\text{SCN})_2$ method, the $\text{HOC}_6\text{H}_4\text{COONa}$ method, and barium sulfate turbidimetry.

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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 NH_4^+ 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

募集案内

平成18・19年度採用分 特別研究員－RPD 募集

(独)日本学術振興会では、特別研究員事業の一環として、優れた若手研究者が出産・育児による研究中断後、円滑に研究現場に復帰する環境を整備するため、研究奨励金を一定期間支給し、研究活動再開を支援する特別研究員－RPDを平成18年度より創設。

今回、平成18年度と平成19年度採用分の募集を同時に行なう。

記

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