Photometric Screening of Tetrabromobisphenol A in Resin Using Iron(III) Nitrate/Hexacyanoferrate(III) Mixture as a Colorimetric Reagent

Hiroyuki YANAGISA W A, Kenji SASAKI, Yoshimi SASAKI, Asumi OMATA, Rina ICHINO, and Shigehiko FUJIMAKI†

Central Chemical Laboratory, SGS Japan Inc., YBP East Tower 12F, 134 Godo-cho, Hodogaya, Yokohama 240-0005, Japan

This study aims to provide a simple way to identify the possibility of tetrabromobisphenol A (TBBPA) present in polymers without the need for complicated separation with expensive equipment. Since the presence of phenolic hydroxyl groups is known to be identifiable by the reduction of Fe$^{3+}$ to Fe$^{2+}$ in a ferric coloring reagent, the possibility of TBBPA being present in a polymer can be screened by a photometric measurement. A mixed solution of iron(III) nitrate and potassium hexacyanide(III) acid was used as a ferric coloring reagent. With this method, the concentration of TBBPA can be estimated from the photometric absorbance corresponding to the depth of the blue color produced by reduction of the ferric reagent in the presence of Fe(NO$_3$)$_3$. The limit of detection (LOD) was determined to be approximately 2 mg/kg using the Student’s t-test (99% confidence), and a reproducibility of approximately 3% was determined by the relative standard deviation (RSD) from measurements of calibration samples ($n = 7$). Furthermore, TBBPA in actual polymer samples was screened without the need for any complex processing steps. Because this colorimetric method measures TBBPA by detecting phenolic groups, it may overestimate the TBBPA concentration in the presence of other similar phenolic substances. Nonetheless, this simple colorimetric method should help to quickly identify the presence of TBBPA in various polymers.

Keywords TBBPA, tetrabromobisphenol A, brominated flame retardants, colorimetric method, additive polymer, hazard identification

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Introduction

In the same way that organic chlorine compounds have various unique functions, organic bromine compounds have also been recognized to have the unique property known as flame retardancy. Flame retardants are substances that are added to products, such as televisions (TVs), personal computers (PCs), carpets, curtains, and housing, materials to make them less flammable in the event of a fire. Among various types of flame retardants, brominated flame retardants (BFRs) have been widely used in many products. Flame-retardant chemicals are often present in amounts as high as 10 to 20 percent of the weight in products for flame retardance. However, many of these retardants were identified as bioaccumulators, and environmental pollution caused by BFRs has become a global problem. A particular concern related to polybrominated diphenyl ethers (PBDEs), which were mainly used in home appliances (plastic housings for TVs and PCs, etc.), and have long been regulated by global chemical laws as a substance that causes serious environmental pollution. These PBDEs were sometimes called the “second polychlorinated biphenyl (PCB)” because of their structural resemblance to PCB (i.e., multiple bromine substituents around two linked benzene rings).

Furthermore, hexabromocyclododecane (HBCDD), another type of BFR, which was widely used to make textile products less flammable, has also been listed as a regulated substance under the Stockholm Convention. Due to the tightening of such global regulations, alternatives to mainstream BFRs have been marketed. For example, phenol-type BFRs, such as tetrabromobisphenol A, are now widely used as an alternative to the conventional BFRs. In fact, TBBPA has become the most produced brominated flame retardant in the world. The European Commission released a final report (Pack 15 Public Consultation) on February 11, 2021 containing recommendations for TBBPA restrictions under the RoHS 2 Directive. The criteria of TBBPA is stated as 1000 mg/kg in the weight of the base metal. Unfortunately, once again, TBBPA has also recently been raised as a subject of concern in the context of global chemical regulation. Under these circumstances, little discussion has been undertaken about TBBPA from a chemical management perspective, including its test methods. TBBPA, like other BFRs, can be quantitatively determined either by gas or liquid chromatography/mass (GC/LC-MS) spectroscopy-based methods. These test methods require complex pretreatment procedures and expert analysis, which are both costly and time consuming. Nonetheless, there is a growing demand for rapid identification of the presence/absence of certain BFRs at a regulatory level. For example, an enzyme-linked immunosorbent assay (ELISA)-like approach has been proposed for measuring TBBPA; however, such a method

Notes

1 To whom correspondence should be addressed.
E-mail: shigehiko.fujimaki@sgs.com
requires the use of expensive enzymes and a specific storage device, and thus is not easily implemented. Under these circumstances, a simple bisphenol A (BPA) test method using a ferric reagent (a mixed solution of iron(III) nitrate and potassium hexacyanide(III) acid) attracted our attention. The reactions that occur when TBBPA is exposed to a ferric solution may also be the key to its identification. Unfortunately, the previous study referred only to BPA without examining the details of the colorimetric reaction pathway. The purpose of this study is to validate the screening method for TBBPA by focusing on color changes caused by the reaction of the ferric reagent with phenolic hydroxyl groups. Here, the presence of phenolic hydroxyl groups is known to be identifiable by the reduction of Fe$^{3+}$ to Fe$^{2+}$ in a ferric coloring reagent, the possibility of TBBPA present in a polymer can be screened by a photometric measurement. With this method, the concentration of phenolic hydroxyl groups can be estimated from the depth of blue color produced by the reduction of the ferric reagent in the presence of Fe(NO$_3$)$_3$ (and subsequent reaction between Fe$^{3+}$ and K$_3$[Fe(CN)$_6$]). The change in absorbance induced by the reaction of the ferric reagent with phenolic hydroxyl groups can be detected with a spectrophotometer. Because this method identifies TBBPA as a compound containing phenolic hydroxyl groups, it can overestimate the TBBPA concentration in the presence of other similar phenolic substances. Nonetheless, this simple colorimetric method should help to quickly identify the presence of TBBPA in various polymers without the need for complicated separation with expensive equipment.

**Materials and Methods**

**Preparation of reagents**

The materials used in this study include the following reagents purchased from Tokyo Chemical Industry Co., Ltd., (Tokyo, Japan): bisphenol A (BPA, 99%), bis(2-ethylhexyl) benzene-1,2-dicarboxylate (DEHP, 99%), tris(2-chloroethyl) phosphate (TCEP, >97%) and tetrabromobisphenol (93%). Other reagents, such as iron(III) nitrate (Fe(NO$_3$)$_3$)-9H$_2$O, 98% and iron(III) hexacyanide (potassium ferricyanide (K$_3$[Fe(CN)$_6$]), 98%) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). In addition, iron(II) chloride tetrahydrate (FeCl$_2$-4H$_2$O, 99%) and ethanol (99.5%) were purchased from Kanto Chemical Co., Inc (Tokyo, Japan) for this study. Ultrapure water (Milli-Q) was used in all experiments. Test tubes (p-15S) prepared for this study were purchased from Nichiden-Rika Glass Co., Inc. (Kobe, Japan). A commercially available acrylonitrile butadiene styrene (ABS) sample containing TBBPA was prepared as a real sample for this study. Polystyrene (PS) particle (average Mw 35000, Sigma-Aldrich Co. LLC), (St. Louis United States) and polycarbonate (PC) plate (thickness 0.2 mm, Sugawara Koge Co., Ltd.) (Tokyo, Japan) were purchased to prepare our own real samples. The filter paper (150-mm diameter, No. 2, circle type) was purchased from ADVANTEC Co. (Tokyo, Japan).

**Calibration procedure**

Calibration standard solutions were prepared with TBBPA in a 50% ethanol/water solution with final concentrations of 0, 2, 6, 11, 20, 23 mg/kg. When measuring using the spectrophotometer, a 1.9 mL aliquot of the sample was placed in an acrylic cuvette (optical length 1 cm). After adding 0.1 mL of a 2% ferric iron coloring reagent (final concentrations 0.1%) to the cuvette, and was held for 2 min, the absorbance at 690 nm was measured. With a UV-Visible double-beam spectrophotometer (Carry 60, Agilent Technologies, Santa Clara, CA, USA). Prior to each measurement, zero calibration was performed with a blank solution at 690 nm. The absorbances were measured with a UV-Visible double-beam spectrophotometer (Carry 60, Agilent Technologies, Santa Clara, CA, USA). As supplementary data, the reagent concentration and the reaction time were optimized with TBBPA at 23 mg/kg. Figures S1(a) and S1(b) (Supporting Information) show that 0.1% ferric coloring reagent is sufficient to complete the reaction in 2 min.

**Sample preparation to confirm selectivity**

A 50% ethanol solution was prepared by mixing ethanol and ultrapure water. Solutions of 100 mg/kg DEHP, 100 mg/kg TCEP, 5 mg/kg BPA, and 15 mg/kg TBBPA were prepared with 50% ethanol. In the same way as in the "Calibration procedure" section, a 1.9 mL aliquot of the sample was placed in an acrylic cuvette (optical length 1 cm). After adding 0.1 mL of the 2% ferric iron coloring reagent (final concentrations 0.1%) to the cuvette, the absorbance at 690 nm was measured after holding for 2 min.

**Preparation of real samples**

A sample containing TBBPA in ABS resin at 8.6 ± 0.58% was cryogenically ground into a powder using liquid nitrogen. A quantity of 7.0 mg of a powdered sample was placed into a test tube and extracted with 2.0 mL ethanol by sonication for 15 min. After removing the polymer sample from the test tube, the sample solution (5 μL) was added to 2 mL of 0.1% ferric iron coloring reagent, and the absorbance was measured at 690 nm after holding the mixed solution for 2 min. Here, the absorbance of 1.0 was converted to a TBBPA concentration of 23 mg/kg. For a comparison with this method, the concentration of TBBPA in the sample was analyzed using gas chromatography (GC-MS) (EGA/Py-3030D, Frontier Lab LTD and GCMS-QP2010 Shimadzu Corporation) by our previously reported method. The type of resin was identified by FT-IR (NICOLET 380, Thermo Fisher Scientific) using a library search (OMNIC 7.3). Polystyrene particles and polycarbonate plates were cryogenically ground into powders, and ultrasonically dissolved into a polymer solution with acetone at a concentration of 5.0 mg/mL (2.0 mL). Then, 100 μL of 1.0% TBBPA in an ethanol solution was added to each polymer solution and dried at room temperature while purging acetone with a spray concentration unit (S-024, EYELA, Tokyo Rikaki Co., Ltd.) (Tokyo, Japan). As mentioned above, both extraction and absorbance measurements were performed on polystyrene particles and polycarbonate plates in the same procedure as for ABS.

**Results and Discussion**

**Reaction of TBBPA with Fe(NO$_3$)$_3$/K$_3$[Fe(CN)$_6$]**

Figure 1 shows a schematic diagram of the reaction pathway for photometric screening of TBBPA using a ferric reagent (Fe(NO$_3$)$_3$/K$_3$[Fe(CN)$_6$]). A mixture of solution of iron(III) nitrate and potassium hexacyanide(III) acid was used as a ferric coloring reagent. The concentration of TBBPA is determined from the change in the absorbance caused by a reduction reaction between Fe$^{3+}$(Fe(NO$_3$)$_3$) and TBBPA. Here, the colorimetric reaction pathway proposed by Park et al. for BPA has been examined, and we verified its applicability to the reaction between TBBPA and a ferric solution. With reference to this result, we came up with a proposal for a reaction pathway that can be applied to identify TBBPA as a compound containing
a phenolic hydroxyl group.

As can be seen in Fig. 2, the presence or absence of a colorimetric reaction between TBBPA and a ferric solution (Fe(NO₃)₃/K₃[Fe(CN)₆]) was confirmed using the spectral absorbance under various conditions. Comparing the four lines in Fig. 2 (a), (b), (c), and (d), a broad peak at 550 – 800 nm only appeared in the presence of TBBPA, Fe(NO₃)₃ and K₃[Fe(CN)₆], with the maximum absorption at 690 nm. Two minutes after adding the reagent, the color gradually changed from brown to dark blue. Further measurements were performed with a mixed solution of Fe²⁺ (FeCl₂) and K₃[Fe(CN)₆] to investigate the difference in the spectral profiles. As is generally well known, when K₃[Fe(CN)₆] is added to a solution of Fe³⁺, the solution turns brown, and when added to a solution containing Fe²⁺, it turns dark blue. As shown with in Fig. 2(e), the spectrum of a mixed solution of TBBPA, Fe(NO₃)₃, and K₃[Fe(CN)₆] was almost the same as that of a mixture of Fe²⁺ (FeCl₂) and K₃[Fe(CN)₆]. From these results, it was found that Fe³⁺ was first reduced to Fe²⁺ by TBBPA and, at the same time, the reaction of Fe²⁺ and K₃[Fe(CN)₆] caused the blue coloration of the solution. Here, the colorimetric reaction pathway proposed by Park et al. specifically for BPA has been validated and confirmed to be applicable to the reaction between TBBPA and ferric solution. Pre- and post-reaction solutions containing TBBPA and ferric reagent were confirmed with GC-MS to clarify how TBBPA changes upon reacting with ferric solution. Selective ion monitoring (m/z 529) data obtained from various solutions using GC-MS are shown in Fig. S2 in Supporting Information. No mass chromatographic peak associated with TBBPA was observed in selective ion monitoring and the total ion chromatogram in GC-MS analysis. The peak attributed to TBBPA disappeared only when Fe(NO₃)₃ was present in the solution. Considering these results, Fe³⁺ (Fe(NO₃)₃) was spontaneously reduced to Fe²⁺ by TBBPA, and this reduction subsequently led to blue coloring by the reaction of Fe²⁺ and K₃[Fe(CN)₆]. The reaction efficiency was almost 100%. However, the substance produced by a further reaction of the free-radicalized TBBPA has not yet been identified.

**Calibration curve**

Calibration was performed with a standard solution of 0 - 23 mg/kg TBBPA to ensure reliable quantitative results, as described in the section above (Calibration procedure). The results are shown in Fig. 3. The results indicate that the absorbance at 690 nm increased linearly as the concentration of TBBPA increased. In other words, Turnbull blue was successfully synthesized in proportion to the concentration of TBBPA. Table 1 summarizes the experimental parameters, such as the sensitivity, signal-to-noise ratio (S/N), relative standard deviation, and correlation coefficient (R²). The limit of detection (LOD) was found to be approximately 2 mg/kg with the
TCEP, 5 mg/kg BPA, and 15 mg/kg TBBPA. Different solutions of 50% ethanol, 100 mg/kg DEHP, 100 mg/kg.

Absorbance after reacting 0.1% Fe(NO$_3$)$_3$/$\text{K}_3[\text{Fe(CN)}_6]$ with 4.

Fig. 4 Absorbance after reacting 0.1% Fe(NO$_3$)$_3$/$\text{K}_3[\text{Fe(CN)}_6]$ with different solutions of 50% ethanol, 100 mg/kg DEHP, 100 mg/kg TCEP, 5 mg/kg BPA, and 15 mg/kg TBBPA.

Student’s $t$-test (99% confidence) by repeating measurements seven times ($n=7$) on a 2 mL sample, and a reproducibility within 2.9% was confirmed by the relative standard deviation (RSD) of the calibration sample. In addition, the correlation coefficient of the calibration curve exceeded 0.99. A sample amount of 2 g would be sufficient to meet the common regulatory criteria of 1000 mg/kg. In this way, TBBPA can be detected easily in a few minutes by simply adding a ferric coloring reaction with the reagent. The results of this study indicate that this method recognizes the target analyte, TBBPA, as one of the phenolic hydroxyl groups. Although this method has limited selectivity for recognizing TBBPA from other phenolic hydroxyl groups, it still should be useful for onsite analysis due to the convenience of being able to analyze by simply adding reagents.

Selectivity

The reaction selectivity was investigated by a comparison with experiments performed in accordance with the method as described in the section above (Sample preparation to confirm selectivity) using 50% ethanol, 100 mg/kg DEHP, 100 mg/kg TCEP, 5 mg/kg BPA, and 15 mg/kg TBBPA. Figure 4 shows the absorbance of the above five substances at 690 nm. A strong absorption at 690 nm was observed in the spectra of TBBPA and BPA solutions. Conversely, absorption at 690 nm was barely observable in solutions with other substances, even at a content of 1%. In addition, any compound containing a phenolic hydroxyl group, such as 2,6-di-tert-butyl-p-cresol and 4,4′-methylenbis (2,6-di-tert-butylphenol), can be subjected to a ferric coloring reaction with the reagent. The results of this study indicate that this method recognizes the target analyte, TBBPA, as one of the phenolic hydroxyl groups. Although this method has limited selectivity for recognizing TBBPA from other phenolic hydroxyl groups, it still should be useful for onsite analysis due to the convenience of being able to analyze by simply adding reagents.

Real sample

The proposed colorimetric method can be used to determine TBBPA without the need for complicated separation methods or expensive equipment, such as GC-MS and high-performance liquid chromatography (HPLC) systems. To confirm the applicability of the proposed colorimetric method to real samples, ABS, PC and PS resins containing TBBPA at concentrations of 8.6 ± 0.58, 10, and 10%, were prepared for this study, respectively. The concentration of TBBPA in commercially available ABS samples has been pre-quantified by the conventional GC-MS method. On the other hand, PC and PS samples were prepared from each plastic powder by adding a specific amount (equivalent to 10%) of TBBPA to a polymer solution dissolved in acetone and drying. Table 2 shows a comparison of results obtained from a real sample using the conventional method and this method. A sample volume of 7 mg was prepared for the analysis to sufficiently evaluate the reference values. The RSD of the method was almost the same as that of the conventional method, and the reproducibility was sufficiently similar. Regarding the accuracy, there were within 20% differences between the conventional method and this method except for PC resin. The concentration of TBBPA obtained from the PC resin was significantly higher than expected due to the influence of the phenolic hydroxyl groups of the base material. Although it is not suitable for quantitative analysis, it was found to be sufficient for onsite screening analysis.

Application to test paper

Following the confirmation of sufficiently low detection limits by a spectrophotometric determination of the sample solution, a visual identification of TBBPA was examined on a test paper for immediate semi-quantitative analysis without the use of a device. Using ethanol as a standard solution, 0, 50, 100, and

| Base material | TBBPA value from GC/MS | Charged amount of TBBPA | Current method |
|---------------|-------------------------|-------------------------|---------------|
| ABS           | 8.6 ± 0.58              | —                       | 11 ± 0.48     |
| PC            | 10                      | 15 ± 1.1                |               |
| PS            | 10                      | 8.8 ± 0.26              |               |

Unit: percentage: Weight of TBBPA in weight of base resin. The estimated TBBPA value for ABS was pre-quantified by conventional GC-MS. The TBBPA values for PC and PS indicate the charged amount values against the weight of the base material.
500 mg/kg samples of TBBPA were prepared in ethanol. An aliquot (90 μL) of these standard solutions was inserted into macro tubes, mixed with 10 μL of a 1% ferric reagent (final conc. 0.1%) in microtubes and held for 2 min. Then, a portion of the solution (20 μL) was dropped on filter paper and taken with a digital camera (DMC-FX07, Panasonic Corporation, Osaka, Japan). As shown in Fig. 5, TBBPA can be qualitatively identified by simply adding a few drops of the test solution onto the filter paper if a spectrophotometer is not available. The presence or absence of TBBPA can be determined by the naked eye at a level of 50 mg/kg or more. The lower limit of visual detection is about 25-times worse than the lower limit of spectrometric detection, but it should be useful for a rough judgment.

Conclusions

The objective of this study was to provide a simple way to identify the possibility of tetrabromobisphenol A (TBBPA) being present in polymers without requiring any complex pretreatment and GC/LC-MS measurements. Since the presence of phenolic hydroxyl groups is known to be identifiable by the reduction of Fe³⁺ to Fe²⁺ in a ferric coloring reagent, the possibility of TBBPA present in a polymer can be screened by a photometric measurement. The change in the absorbance induced by the reduction (Fe³⁺ to Fe²⁺) was detected with a spectrophotometer. The LOD was determined to be approximately 2 mg/kg by the Student’s t-test (99% confidence) by repeating the measurement seven times and approximately a 3% reproducibility was confirmed by RSD (n = 7). Furthermore, the spectrophotometric screening of TBBPA in actual plastics has been demonstrated on ABS, PC, and PS resin samples. The concentrations of TBBPA in real samples obtained using the spectrophotometer were almost consistent with a value pre-quantified by conventional GC-MS or the values compounded in the samples. Because this method identifies TBBPA as a compound containing phenolic hydroxyl groups, it can overestimate the TBBPA concentration in the presence of other similar phenolic substances. In fact, the concentration of TBBPA obtained from the PC resin was significantly higher than expected due to the influence of the phenolic hydroxyl groups of the base material. Despite this limitation, this colorimetric approach can be applied to screen for TBBPA concentrations without the need for a complex separation with expensive equipment.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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