

## **Simple colorimetric determination of phthalates in polymers by dye formation**

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## **Abstract**

This paper describes a simple method for quantifying phthalates via the conversion of anhydrous phthalates to a dye. The phthalate was hydrolyzed with sodium hydroxide and dehydrated to form phthalic anhydride, which was converted to a marker, namely fluorescein, by reaction with resorcinol. Concentrated sulfuric acid was used as the catalyst. The presence of a phthalate was determined by absorbance spectrophotometry. The detection limit of this method for di(2-ethylhexyl) phthalate was 0.1  $\mu\text{mol}$ , and the relative standard deviation with respect to reproducibility was approximately 10% ( $n = 3$ ). For other phthalates, namely diisobutyl phthalate, dibutyl phthalate, bis(butylbenzyl) phthalate, di-*n*-octyl phthalate, diisononyl phthalate, and diisodecyl phthalate, the absorbance deviation was less than 20%, which is within the acceptable range for screening purposes. Because the phthalates were hydrolyzed to enable colorimetric determination, the identification of individual phthalates is beyond the scope of this method. However, this simple colorimetric method can easily be used to determine the total amount of phthalates in real samples at the sub-micromolar level, by converting the phthalates to dyes. The applicability of this method was investigated by analyzing actual samples containing various phthalates. The quantitative results were almost the same as those obtained by using a conventional gas chromatographic method.

**Keywords:** Phthalate, Colorimetric, Fluorescein, Dye, Screening, Polymer, Additive, Plasticizer, IEC, RoHS, Toy safety

## 1 Introduction

Recently, the adverse effects of phthalates on human health have become an issue of increasing concern. Phthalates are extensively used in industry as plasticizers and can be present in high concentrations in polymer-based products such as cable insulation, wallpaper, food packaging, and medical and childcare products.<sup>1,2</sup> Some phthalates that pose a threat to the reproductive system (e.g., birth defects) are currently classified as major hazardous substances and are also suspected to have endocrine-disrupting properties.<sup>3</sup>

The use of these compounds has therefore been restricted in some products such as medical and childcare products, and stricter controls are expected to be enforced. In general, such additives are undetectable without performing detailed analyses. The presence of restricted additives is commonly determined by conventional gas chromatography-mass spectrometry (GC-MS) and this requires complicated pre-treatment procedures such as solvent extraction and clean-up procedures, which are usually expensive and time consuming.<sup>4</sup> The use of screening to reduce the complexity of GC-MS analysis has therefore attracted considerable attention. A convenient screening method has been published by the International Electrotechnical Commission (IEC). The method is based on pyrolysis/thermal desorption gas chromatography-mass spectrometry (Py/TD-GC-MS) and is part of the IEC-62321 family of standards (part 8).<sup>5</sup> The Py/TD-GC-MS method enables solvent-free direct sample injection without the need for complicated pre-treatment procedures.<sup>6-8</sup> However, in an actual business scenario, a simple screening method that enables manufacturers to verify the ingredients of various materials on a daily basis is needed. For example, Fourier- transform infrared (FTIR)

spectroscopy enables the nondestructive analysis of phthalates,<sup>9,10</sup> but the detection limits are usually considerably higher than the regulatory criteria and FTIR spectroscopy cannot be used for performing compliance assessment. An alternative method involves a colorimetric approach. A previous study investigated colorimetric screening of phthalates on the basis of nanoparticle aggregation.<sup>11</sup> However, the need to use specially treated gold nanoparticles is a major drawback of previously proposed colorimetric methods.

In this study, we developed a simple colorimetric method that uses specific reaction steps for selectively converting phthalates to dyes. Fluorescein, which is the main dye used in this study, was synthesized from phthalates by using concentrated sulfuric acid as the catalyst.<sup>12</sup> The phthalates can be quantified spectrophotometrically from the absorbance of fluorescein, which is a suitable marker, in the visible-light region. The objective of this study was to develop a simple method for screening phthalates in various polymers on the basis of color changes induced by a specific reaction, without the use of conventional labels such as enzymes,<sup>13</sup> liposomes,<sup>14</sup> and quantum dots.<sup>15</sup> The phthalates in the samples were extracted with ethanol and dehydrated after hydrolysis with sodium hydroxide to produce phthalic anhydride. The phthalic anhydride was converted to a dye (i.e., fluorescein), which acted as a marker for phthalates. This colorimetric method positively identified the presence of phthalates in sample solutions at levels well below the regulatory limits. For example, a detection limit of approximately 0.1  $\mu\text{mol}$  was obtained for di(2-ethylhexyl) phthalate (DEHP). With respect to reproducibility, a relative standard deviation of approximately 10% ( $n = 3$ ) was confirmed after optimization of the test method. In this study, phthalates were hydrolyzed for colorimetric determination. The identification of individual phthalates is not within the scope of this method. However, this colorimetric method provides a

simple screening approach for the determination of phthalates at the regulatory level without the need for complicated separation using expensive equipment.

## 2 Materials and methods

### 2.1 Reagents

The reagents used in this study, i.e., resorcinol, phthalic anhydride, sodium hydroxide, ethanol, hexane, 2-propanol, acetone, concentrated sulfuric acid, concentrated hydrochloric acid, *o*-dimethyl phthalate, *m*-dimethyl phthalate, *p*-dimethyl phthalate, diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), bis(butylbenzyl) phthalate (BBP), DEHP, di-*n*-octyl phthalate (DNOP), diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), dioctyl adipate (DOA), tricresyl phosphate (TCP), and acetyl tributyl citrate (ATBC), were purchased from the Kanto Chemical Co., Inc. (Tokyo, Japan). The reagents were all analytical grade. Ultrapure water (Milli-Q) was used in all experiments. Test tubes (p-15S) were purchased from the Nichiden-Rika Glass Co., Inc. (Kobe, Japan). Reference materials (RM), which were originally developed for the calibration of Py/TD-GC-MS and contained  $20 \pm 4$   $\mu\text{mol/g}$  of phthalates (P/NS225-31003-91), were purchased from the Shimadzu Corp., (Kyoto, Japan) and used for cross-validation with the GC-MS screening method.

### 2.2 Instrumentation

Target analytes were extracted at room temperature with ethanol in an ultrasonic bath (Ultrasonic Cleaner model T-9, L & R). A dry thermo unit (DTU-1C, Taitec

Corporation, Koshigaya, Japan) was used for dehydration. The test solutions were inserted into a quartz cell (optical length 1.0 cm) and absorbances were determined with a UV-visible double-beam spectrophotometer (Carry 60, Agilent Technologies, California, USA). The results were verified by using a GC-MS system (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) coupled with a pyrolyzer/thermal desorption unit (PY-3030D, Frontier-Lab, Koriyama Japan). The instrument settings were the same as those used in previous studies.<sup>6,7</sup>

### 2.3 Synthesis of fluorescein from phthalates

The phthalates were hydrolyzed to phthalic acid with sodium hydroxide and dehydrated using the dry thermo unit. After dehydration, fluorescein was synthesized by the selective reaction between phthalic anhydride and resorcinol with concentrated sulfuric acid as the catalyst. The presence of phthalates was colorimetrically determined with a spectrophotometer. The reaction steps, i.e., hydrolysis, dehydration, and fluorescein synthesis, are summarized in Fig. 1.

### 2.4 Colorimetric determination of fluorescence

The phthalate concentrations were determined from the molar absorption coefficient of fluorescein at 494 nm ( $\varepsilon = 76900 \text{ mol cm/L}$ ).<sup>16</sup> Scanning was performed in the wavelength range 200 to 600 nm at a scanning speed of 600 nm/min. Zero calibration was achieved at 600 nm before each measurement.

## 2.5 Calibration procedure

Calibration standard solutions were prepared by adding 0, 0.05, 0.12, 0.25, 1.24, 2.48, and 4.96  $\mu\text{mol}$  of DEHP to ethanol (2 mL). Sodium hydroxide (1 M, 1.5 mL) was added to the standard solution to hydrolyze DEHP. The mixed solution was sonicated for 15 min to promote hydrolysis. After hydrolysis, the solution pH was adjusted to less than 1 by adding concentrated hydrochloric acid (250  $\mu\text{L}$ ), then the sample was dehydrated at 125  $^{\circ}\text{C}$  to produce phthalic anhydride. Fluorescein was synthesized by adding resorcinol (15 mg, 130  $\mu\text{mol}$ ) and concentrated sulfuric acid (100  $\mu\text{L}$ ) to the mixed solution and then heating at 125  $^{\circ}\text{C}$  for 10 min. The mixture was cooled to room temperature and ethanol (2 mL) was added to induce precipitation. Several microliters of the supernatant were collected and mixed with sodium hydroxide (1 M, 2.0 mL) to adjust the solution pH ( $\text{pH} > 10$ ). The absorbance of the standard solution was determined spectrophotometrically and a calibration curve was constructed. Similar procedures were performed with phthalates with various alkyl chain lengths (i.e., DIBP, DBP, BBP, DNOP, DINP, and DIDP).

## 2.6 Quantification of phthalates in real samples

A polyethylene sample (100 mg) containing  $20 \pm 4$   $\mu\text{mol/g}$  of phthalates was placed in a test tube. Ethanol (2.0 mL) was added and ultrasonic extraction was performed for 30 min. After removing the polymer sample from the test tube, the extracts were processed and used to synthesize fluorescein, as described above. The concentration of fluorescein, which served as a marker for phthalates, was determined from the absorbance at 494 nm. An absorbance of 1.0 corresponded to a fluorescein

concentration of 13.0  $\mu\text{mol/L}$ . The net amount of phthalates was calculated on the basis of the synthetic efficiency and dilution ratio.

### 3 Results and Discussion

#### 3.1 Synthesis of fluorescein from phthalic anhydride

The efficiency of fluorescein synthesis with respect to the amount of phthalic anhydride was determined. Fluorescein was synthesized by the selective reaction of phthalic anhydride (0, 8.50, 30.7, 81.6 and 137  $\mu\text{mol}$ ) with resorcinol (130  $\mu\text{mol}$ ) using concentrated sulfuric acid (100  $\mu\text{l}$ ) as the catalyst. The presence of phthalate was determined colorimetrically in a solution diluted 300-fold (Fig. 2(a)) or 340-fold (Fig. 2(b)) using a spectrophotometer. The pH dependency was confirmed by synthesizing fluorescein under two conditions ( $\text{pH} < 1$  and  $\text{pH} > 12$ ) adjusted with conc. HCL and NaOH. Figure 2(a) shows the relationship between the amount of phthalate anhydride and the fluorescein absorbance. The spectra of the samples containing fluorescein synthesized by the reactions of phthalic anhydrides and resorcinol are shown in Fig. 2(b). The absorbance at 494 nm increased with increasing amount of phthalic anhydride (0 to 135  $\mu\text{mol}$ ) under acidic conditions. The signal at 494 nm shows that fluorescein was successfully synthesized when sufficient phthalic anhydride was present. However, under alkaline conditions, the characteristic absorption of fluorescein at 494 nm was not observed. The data provide convincing evidence that fluorescein cannot be easily synthesized from phthalic anhydride under alkaline conditions. Phthalic acid has  $\text{pK}_a$  values of 2.89 and 5.51,<sup>17</sup> therefore at high pH values sodium hydrogen phthalate or sodium phthalate is formed. Alkaline conditions suppress the formation of phthalic anhydride. Table 1 summarizes the sensitivities, relative standard deviations (RSDs), correlation coefficients, and synthetic efficiencies under



acidic and alkaline conditions. These results indicated that sufficiently low pH is desirable to synthesize fluorescein from phthalic anhydride and resocinol. Fluorescein was synthesized from phthalic anhydride under acidic conditions with a sufficiently high reproducibility, despite the relatively low synthetic efficiency of 8.0%. This reaction step therefore provides a simple method for screening phthalates, without the need for complicated separation and purification steps.

### 3.2 Selectivity for *ortho* phthalates

Phthalates consist of *ortho*, *meta*, and *para* isomers. Currently, only *ortho* phthalates are subject to regulations. The reaction selectivity was investigated by comparing the results of experiments performed with *o*-, *m*-, and *p*-DMP (4.96  $\mu$ mol) in the same operation as “calibration procedure”. The final solution was diluted 12-fold with 1 M sodium hydroxide and the absorbances were measured with a spectrophotometer. Figure 3 shows the spectra of *o*-, *m*-, and *p*-DMP after use in this method. A strong absorption at 494 nm was observed in the spectrum of *o*-DMP. Only *ortho* phthalic acids contain carboxyl groups in adjacent positions, and this enables dehydrative intramolecular condensation. Phthalic anhydride, which is used in the Friedel–Crafts reaction with resorcinol, therefore cannot be derived from *meta* and *para* phthalates. The selectivities of DOA, TCP, and ATBC, which are used as plasticizers in polymers, were also determined. No absorption bands at 494 nm were present in the spectra of these three plasticizers (Fig. S1 and Table S1), for the same reason as described above. This indicates that the *ortho* phthalic structure selectively contributes to fluorescein synthesis.

### 3.3 Optimization of hydrolysis conditions

2-Propanol, ethanol, hexane, and acetone were used as organic solvents for optimizing the extraction and hydrolysis steps. In general, a phthalate in a polymer can usually be easily extracted with these solvents. The hydrolysis step was examined to identify the most suitable organic solvent. The experiment was performed by replacing ethanol with the above-mentioned solvent in the operation of "calibration procedure". Figure 4 shows the absorbances of fluorescein samples synthesized from 25  $\mu\text{mol}$  of DEHP using various organic solvents. The figure shows that the order of the hydrolytic efficiencies was 2-propanol > ethanol > direct reaction > hexane > acetone. Here, "direct reaction" indicates the direct reaction of DEHP with sodium hydroxide. These results show that DEHP hydrolysis proceeds smoothly in alcohol solvents, which mix easily with sodium hydroxide solution. However, acetone, which can also be easily mixed with sodium hydroxide solution, is not a suitable solvent for hydrolysis because it can trigger other reactions. Because of its versatility,<sup>18</sup> ethanol is a more suitable hydrolysis solvent than 2-propanol.

### 3.4 Calibration based on hydrolyzability

Calibration was performed with standard solutions containing 0–5  $\mu\text{mol}$  DEHP to ensure reliable quantitative results in the same operation as "calibration procedure". The final solution was diluted 17-fold with 1 M sodium hydroxide and the absorbances were measured with a spectrophotometer; the results are shown in Fig. 5. Fluorescein was successfully synthesized

proportional to the amount of DEHP. Experimental parameters such as the sensitivity, RSD, correlation coefficient, and synthetic efficiency are summarized in Table 2; the data show a sufficiently low detection limit (approximately 0.1  $\mu\text{mol}$ ), high correlation coefficient (0.998), and satisfactory RSD (6.9%). For a polymer sample of mass 1 g, the DEHP detection limit was  $40 \pm 3$  mg/kg, which is lower than the detection limit set in IEC 62321 (50–100 mg/kg).<sup>5</sup> These data indicate that sufficiently extracted phthalates can be detected at levels below the regulatory limit. As shown in Table 2, synthetic efficiency of fluorescein from DEHP was 7.7%. Considering the synthetic efficiency from phthalic anhydride, it was found that 96% hydrolysis was achievable. In the subsequent step, the reaction selectivity was examined by preparing the same amounts of various phthalates. Figure 6 shows that there were no differences between the absorbances of DIBP and DBP. However, the absorbances of BBP, DEHP, DNOP, DINP, and DIDP were approximately 20% lower than those of DIBP and DBP because the hydrolytic efficiency decreased with increasing alkyl chain length. Although the synthetic yield of fluorescein differs by approximately 20% depending on the type of phthalate, the amount of phthalates was assessable in practice

### 3.5 Comparison with results obtained by conventional method for real sample

The proposed colorimetric method can be used to determine the total amount of phthalates without the need for complicated separation methods and use of expensive equipment such as GC-MS and HPLC systems. To confirm the applicability of the proposed colorimetric method to actual samples, a polyethylene sample containing phthalates at a concentration of  $20 \pm 4$   $\mu\text{mol/g}$  was used as a reference material. The data in Table 3 show that the quantitative result delivered by the colorimetric method was comparable to that for the sum of phthalates obtained by using the

conventional Py-GC-MS technique. Considering the efficiency of fluorescein synthesis, the majority of the phthalates would have been sufficiently extracted with ethanol, which would enable quantification of the total amount of phthalates in the polyethylene sample. The regulatory criterion is set at 1000 mg/kg. For safety, the screening results should be assessed by converting “ $\mu\text{mol/g}$ ” to “mg/kg”, on the assumption that the phthalic anhydride or phthalate will have a low molecular weight.

#### 4. Conclusion

The objective of this study was to develop a simple method for screening phthalates in various polymers on the basis of the color changes that are induced by reactions with specific reagents. Ethanol was identified as a suitable solvent for both the extraction and hydrolysis procedures in this method. The selective detection of *ortho* phthalates was observed during optimization. The detection limit ( $0.1 \mu\text{mol}$ ) and RSD (approximately 10%) for DEHP were determined. The absorbance deviations were less than 20% for DIBP, DBP, BBP, DEHP, DNOP, DINP, and DIDP. This colorimetric method identified the presence of phthalates in a standard solution at levels well below the regulatory limit. Because phthalates have to be hydrolyzed to enable colorimetric determination, the identification of individual phthalates is not within the scope of this method. Despite this limitation, this colorimetric method provides a simple screening approach for the determination of phthalates at the regulatory levels without the need for complicated separation with expensive equipment. The quantitative results obtained using the colorimetric method were comparable to those for the sum of phthalates in real samples (polyethylene) determined by a conventional Py-GC-MS technique.

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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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Table 1 Sensitivities, RSDs, correlation coefficients, and synthetic efficiencies of fluorescein synthesized from phthalate under acidic and alkaline conditions.

Conditions	Sensitivity ( $A_{494}/\mu\text{mol}$ )	RSD (%)	Correlation coefficient	Synthetic efficiency (%)
Acidic	$7.6 \times 10^{-3}$	11%	0.998	8.0
Alkaline	$0.2 \times 10^{-3}$	6%	-	<0.003

Table 2 Experimental parameters in DEHP analysis.

Sensitivity ( $A_{494}/\mu\text{mol}$ )	<i>S/N</i> ratio	DL	RSD (%)	$R^2$	Synthetic efficiency (%)
4.2	96	0.1 $\mu\text{mol}$	6.9	0.998	7.7

DL: Detection limit;  $R^2$ : correlation coefficient.

Table 3 Comparison of results obtained with current method and conventional method using reference material (RM).

RM	Reference value	Py-GC-MS	Our assay
RM sheet for Py-GC-MS	$20 \pm 4$	$20 \pm 1$	$18 \pm 2$

Unit:  $\mu\text{mol/g}$ : amount of phthalate ( $\mu\text{mol}$ ) contained in base polymer (g). Standard deviation is based on three measurements.

## Figure Captions

Figure 1 Reaction flow chart for dehydration of phthalates and dye formation. Phthalates were hydrolyzed to phthalic acid with sodium hydroxide (step a to b) and dehydrated with a dry thermo unit (step b to c). After dehydration, fluorescein was synthesized by selective reaction of phthalic anhydride and resorcinol with concentrated sulfuric acid as the catalyst (step c to d). The presence of phthalate was colorimetrically determined with a spectrophotometer.

Figure 2 (a) Relationship between amount of phthalic anhydride and absorbance at 494 nm. Results obtained under acidic and alkaline conditions are shown by open and closed circles, respectively. Preparation amount of phthalic anhydride: 0, 8.50, 30.7, 81.6 and 137  $\mu\text{mol}$ , where the amount of resorcinol and conc.  $\text{H}_2\text{SO}_4$  added is constant at 130  $\mu\text{mol}$  and 100  $\mu\text{l}$ , respectively. The final solution was diluted 300-fold with 1 M sodium hydroxide. (b) Spectra of samples obtained by reaction of phthalic anhydride and resorcinol (Diluted 340-fold in the same procedure as in Fig. 2(a)). Black line: acidic conditions, gray line: alkaline conditions, broken line: blank (1 M sodium hydroxide).

Figure 3 Spectral comparison of three types of resorcinol-spiked DMPs (4.96  $\mu\text{mol}$ ). Broken black line: *o*-DMP, gray line: *m*-DMP, black dashed line: *p*-DMP. The final solution was diluted 12-fold with 1 M sodium hydroxide.

Figure 4 Comparison of absorbance processed in various extraction solvents. Efficiency of DEHP (25  $\mu\text{mol}$ ) hydrolysis was evaluated from the absorbance of fluorescein at 494 nm. Absorbance values were normalized to the value for 2-propanol.



Figure 5 Calibration curve for DEHP (Preparation amount: 0, 0.05, 0.12, 0.25, 1.24, 2.48, and 4.96  $\mu\text{mol}$ ). The absorbances were measured by diluting the final solution 17-fold with 1 M sodium hydroxide.

Figure 6 Dependence on absorbance at 494 nm for each type of phthalate (alkyl chain lengths of DIBP, DBP, BBP, DEHP, DNOP, DINP, and DIDP differ). The collected amount of all the phthalates was 4.96  $\mu\text{mol}$ . Vertical axis of the graph was normalized with the value of DIBP as 1.0. Error bars represent one standard deviation for the three measurements.

## Figures

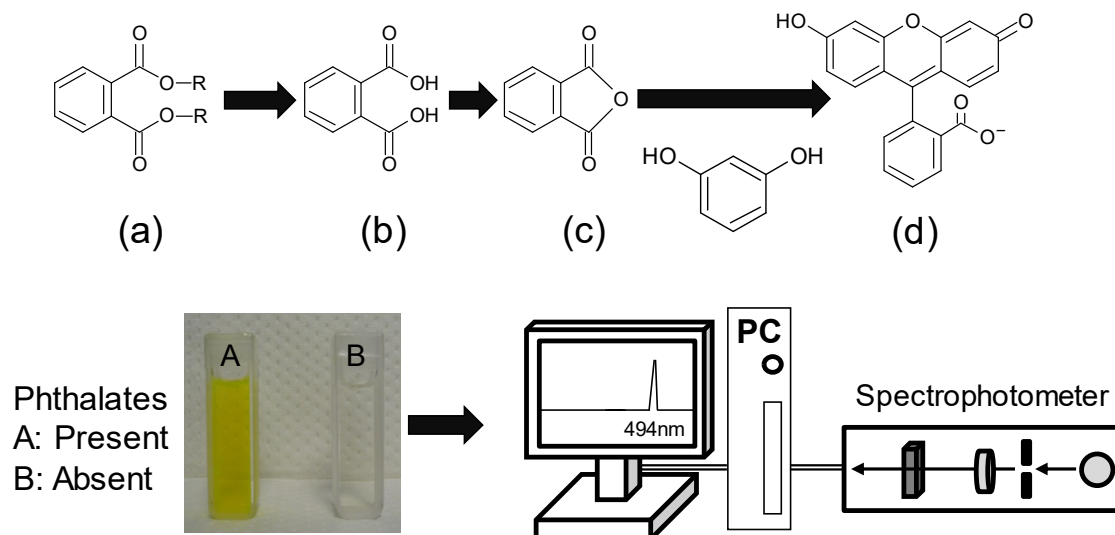


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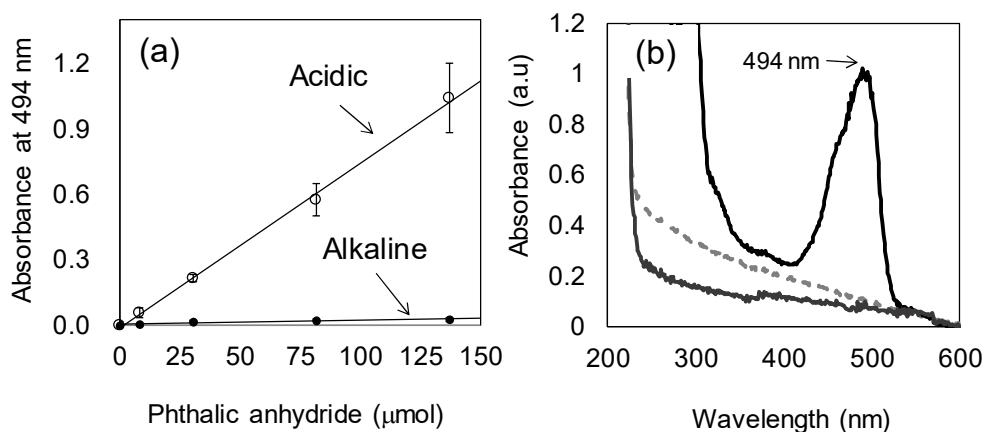


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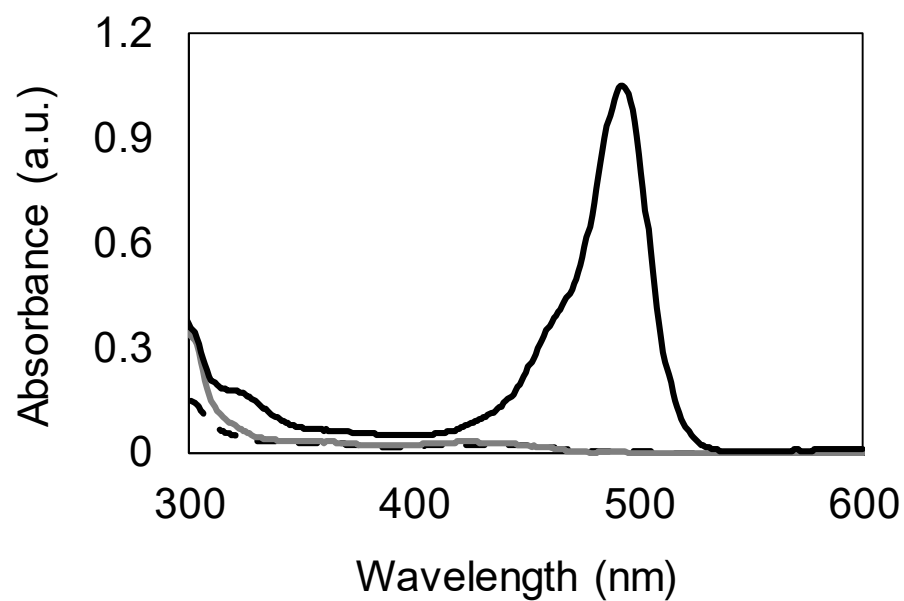


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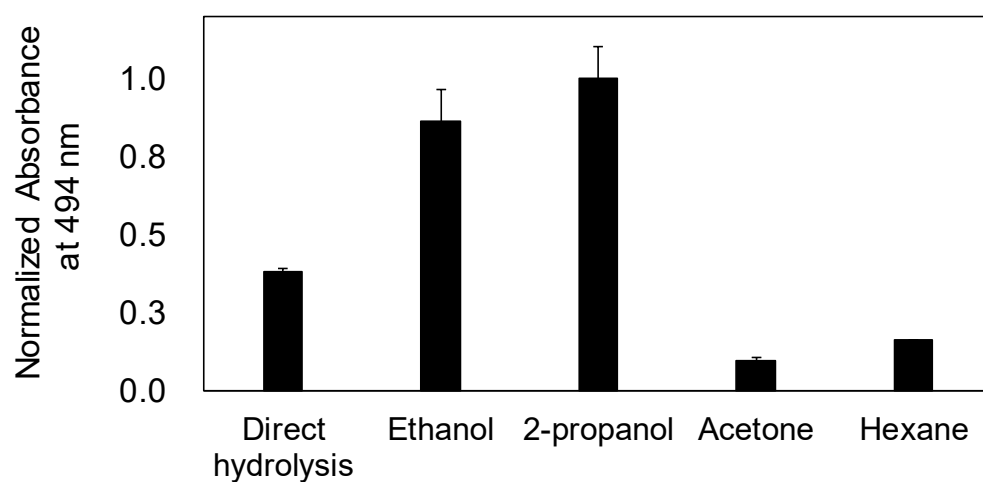


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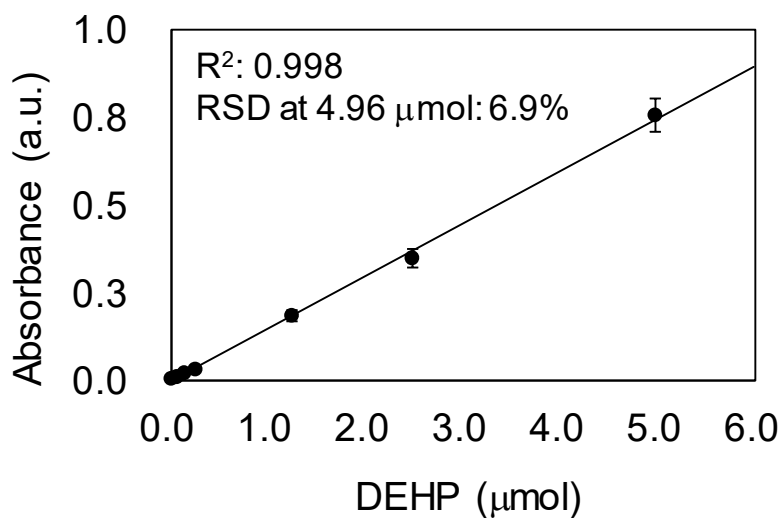


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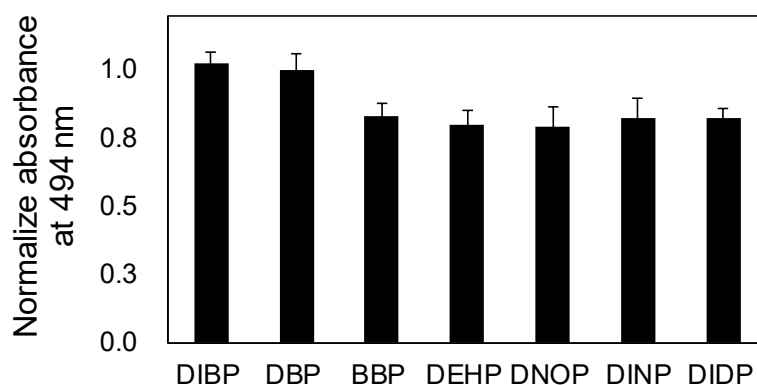


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## Graphical Index

