Fluorometric Quantitative Analysis of Al(III) Ion Using 5-Methoxy-2-phenyliminomethylphenol

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A novel Schiff base ligand (N, O system) 5-methoxy-2-phenyliminomethylphenol (5-CH3O-PMP) was synthesized. Using the synthesized ligand as a fluorescent reagent, a fluorometric method was developed for the quantitative analysis of Al(III) ion. The quantitative analysis of Al(III) ion was performed by making the complex compound between Al(III) ion and 5-CH3O-PMP in ethanol-water solution (85/15, v/v, pH 6.2). The excitation wavelength (λem) of the complex compound was 397 nm while the emission wavelength (λem) was 498 nm. The quantitative analysis of Al(III) ion was carried out by estimating the fluorescence intensity. The various calibration curves were used for the quantitative analysis in the range of 0.27~27 ng/mL Al(III) ion concentrations. The detection limit was 0.027 ng/mL. Using the fluorometric method developed in this study, satisfying results were obtained from various samples such as tap water, hot spring water, river water, sea water and waste water, which contained considerable amounts of interfering ions.

Key Words: Fluorescence spectra, Schiff base ligand, Al(III) Analysis in natural water

Introduction

Toxic Al(III) ion can exist in natural water and ground water. In tap water, a trace of Al(III) ion can also be present due to poly aluminium hydroxychloride or aluminum sulfate which are used as cohesives agents. Al(III) ion, which can be extracted from teapot1 or kitchen utensils,2 is harmful to the human body. Thus, development of quantitative analysis for Al(III) ion is of great interest.

There are many quantitative analytical methods to detect Al(III) ion in aqueous solution. The atomic absorption spectrophotometric method has been used but it has a low detection limit.3-5 Although the fluorometric method is more sensitive than absorption, it has not been widely used except for some organic compounds.

For quantitative analysis of very small amount of Al(III) ion in aqueous solution (ug/mL ~ ng/mL), various types of Schiff base ligands, which contain nitrogen and oxygen atoms,6-11 are used as luminous agents when performing quantitative analysis of a very small amount of Al(III) ion in aqueous solution (ug/mL ~ ng/mL). Recently, several Schiff base ligands were synthesized and used for fluorometric12 and electrochemical sensors13,14 for Al(III) ion. However, fluorometric quantitative method for Al(III) ion has rarely been reported.

In this research, a novel Schiff base ligand 5-methoxy-2-phenyliminomethylphenol (5-CH3O-PMP) was synthesized and used as a luminous agent of Al(III) ion. Using the newly synthesized 5-CH3O-PMP, the amount of Al(III) ion in aqueous solution was measured within the limit of 0.27 ng/mL. The amount of Al(III) ion in tap water, hot spring water, river water, sea water and waste water was analyzed by a newly developed Al(III) ion fluorometric method and compared with the previously reported atomic absorption spectrophotometric method.

Key Words: Fluorescence spectra, Schiff base ligand, Al(III) Analysis in natural water

Reagents and Apparatus. 2-Hydroxy-4-methoxybenzaldehyde, aniline and other metal salts used in this experiment were purchased from Sigma-Aldrich (Missouri, USA, purum grade). Organic solvents such as ethanol, methanol and acid/base agents purchased from Fluka (Buchs, Switzerland, purum grade) were used without further purification. IR spectrum was obtained through Shimadzu IR 440 (Shmadzu, Japan). Fluorescence spectrum was measured with Jasco FP-6300 (Jasco, Japan). 1H-NMR and 13C-NMR spectra were obtained by Varian Mercury 300 NMR (Varian, USA). C.H.N analysis was performed by Valio EL Analyzer (Elementar, Germany). pH was measured by Metrotom 692 pH Meter(Metrohm, Swiss). Distilled water used in this experiment was obtained from Milli-Q plus Hyper-pure distilled water maker(Millipore, USA).

Composition of Agent. To synthesize Schiff base ligand (N, O system), prepare 3 volumetric flasks. In each flask, add 0.02 mole (1.9 g) of aniline and 200 mL of ethanol. Place the solution into chemical reactors (with Liebig condenser) and stir the solution for 10 minutes in 60 °C. Pour 0.2 moles of 2-hydroxy-3-methoxybenzaldehyde (3.04 g), 2-hydroxy-4-methoxybenzaldehyde (3.04 g) and 2-hydroxy-5-methoxy-
benzaldehyde (2.6 mL) each into 3 separate reactors by using separated funnels. The solution turns an orange color. Let the orange color solution react for 6 hours and put the solution in a refrigerator for a day. Then yellow crystals appear. Recrystallize the yellow crystal in methanol and dry the crystal in the vacuum desiccator. A synthetic scheme and chemical composition are described.

**4-Methoxy-2-phenyliminomethylphenol (4-CH$_3$O-PMP).**

Yield: > 68%. Anal. Found: C, 72.91; H, 5.70; N, 6.81; O, 14.58. Calc. for C$_{14}$H$_{13}$NO: C, 72.96; H, 5.66; N, 6.80; O, 14.80%. IR (KBr, cm$^{-1}$): 3200 (v, O-H), 1610 (v, C=N), 1585, 1485 (C=C), 1670 (v, C-O). $^{1}H$-NMR (CDCl$_3$, ppm) 3.93 (s, 3H), 6.90 (d, $J=2.7$, 1H), 6.99 (t, $J=2.4$, 1H), 7.31 (t, $J=6.9$, 5H), 7.42 (t, $J=7.8$, 1H), 8.58 (s, 1H). $^{13}$C-NMR (CDCl$_3$, ppm) 55.4, 101.0, 113.0, 120.9, 126.3, 129.4, 152.2, 161.4, 163.9, 164.0. Mass (EI): m/z: 227 (M$^+$), 77 (fragment).

**5-Methoxy-2-phenyliminomethylphenol (5-CH$_3$O-PMP).**

Yield: > 65%. Anal. Found: C, 79.12; H, 6.18; N, 7.01; O, 7.69. Calc. for C$_{14}$H$_{13}$NO: C, 79.60; H, 6.20; N, 6.63; O, 7.57%. IR (KBr, cm$^{-1}$): 3200 (v, O-H), 1612 (v, C=N), 1585, 1485 (C=C), 1670 (v, C-O). $^{1}H$-NMR (CDCl$_3$, ppm) 3.81 (s, 3H), 6.90 (d, $J=2.7$, 1H), 6.99 (t, $J=2.4$, 1H), 7.31 (t, $J=6.9$, 5H), 7.42 (t, $J=7.8$, 1H), 8.58 (s, 1H). $^{13}$C-NMR (CDCl$_3$, ppm) 55.9, 115.3, 118.0, 118.7, 120.4, 121.1, 126.9, 129.4, 152.2, 155.3, 162.3. Mass (EI): m/z: 227 (M$^+$), 93 (fragment). 77 (fragment).

**6-Methoxy-2-phenyliminomethylphenol (6-CH$_3$O-PMP).**

Yield: > 75%. Anal. Found: C, 79.12; H, 6.18; N, 7.01; O, 7.69. Calc. for C$_{14}$H$_{13}$NO: C, 79.60; H, 6.20; N, 6.63; O, 7.57%. IR (KBr, cm$^{-1}$): 3200 (v, O-H), 1612 (v, C=N), 1585, 1485 (C=C), 1670 (v, C-O). $^{1}H$-NMR (CDCl$_3$, ppm) 3.93 (s, 3H), 6.88 (t, $J=7.8$, 1H), 7.00 (t, $J=7.8$, 1H), 7.28 (t, $J=3.0$, 5H), 7.42 (t, $J=7.5$, 1H), 8.62 (s, 1H). $^{13}$C-NMR (CDCl$_3$, ppm) 56.1, 114.7, 118.5, 119.1, 121.1, 123.7, 123.7, 126.9, 129.4, 148.1, 148.5, 151.4, 162.6. Mass (EI): m/z: 227 (M$^+$), 93 (base). 77 (fragment).

**Experimental Method.** A ligand 5-CH$_3$O-PMP was successfully synthesized. 1.0 $\times$ 10$^{-3}$ M solution of AIK(SO$_4$)$_2$·12H$_2$O was used as the Al(III) ion standard solution and was diluted when necessary. The pH buffer solution of various concentration was prepared from the mixture of acetic acid and salt. Ethanol-water-solvent (85/15, v/v) was used in the preparation of the interfering ion concentration was done by the fluorometry (several metal cation and anion solutions were added to a test solution). Tap water and sea water were investigated. The HNO$_3$ (2 mL/L) solution was added to the test solution.

**Result and Discussion.**

**Synthesis.** Schiff's base ligand 4-CH$_3$O-PMP, 5-CH$_3$O-PMP and 6-CH$_3$O-PMP were synthesized through the following process: Amline, 2-hydroxy-3-methoxybenzaldehyde, 2-hydroxy-4-methoxy-benzaldehyde, 2-hydroxy-5-methoxy-benzaldehyde were dissolved in methanol and the reaction proceeded at 60 °C. C, H and N atomic analysis shows composition of each ligand exactly matched the theoretical values. Imine(-C=N)-'s IR absorption peak, which is characteristic of CH$_3$O-PMP Schiff's base ligands, appeared at 1610-1612 cm$^{-1}$. Hydrogens attached to the carbon of imine group appeared at 8.58-8.62 ppm in $^{1}H$ NMR spectrum. In $^{13}$C NMR spectrum, carbon atoms of imine group were confirmed at 162.3-164.0 ppm. In GC-mass spectrum, m/z value of 231 peak(molecular ion peak, M$^+$) appeared in all ligands and m/z value of 77 peak corresponding to benzene were observed. These results are not illustrated in the figure.
fermed the synthesis of 4-CH₃O-PMP, 5-CH₃O-PMP and 6-CH₃O-PMP, in which the sites of the methoxy groups are all different.

**Fluorescence Spectra.** In Fig. 1, the UV/Vis absorption spectra of 4-CH₃O-PMP (1a), 5-CH₃O-PMP (2a), 6-CH₃O-PMP (3a) are shown as dotted lines. The UV/Vis absorption spectra of Al(III) complex of Al(III)-4-CH₃O-PMP (1b), Al (III)-5-CH₃O-PMP (2b), Al (III)-6-CH₃O-PMP (3b) are shown as solid lines. In addition, the fluorescence spectra of ligands (1A, 2A, 3A) and complexes (1B, 2B, 3B) are shown. As shown in Fig. 1, absorption peaks are observed at 313 nm (6-CH₃O-PMP), 366 nm (4-CH₃O-PMP) and 414 nm (5-CH₃O-PMP) respectively. The ligand 5-CH₃O-PMP exhibits the most red-shifted peak. This is because the conjugation takes place in different ways depending on the position of the aniline double bond and the 4, 5, 6 methoxy group of the phenol compound. Briefly, the methoxy group of 5-CH₃O-PMP is located in a better position for conjugation than that of 4-CH₃O-PMP and 6-CH₃O-PMP. The absorption peaks of 5-CH₃O-PMP were 330 nm and 414 nm. The fluorescence emission intensity from 414 nm was larger than that from 330 nm. Therefore, the emission intensity was measured at 498 nm (λem) using 414 nm as an excitation wavelength.

The fluorescence peak positions of ligands 4-CH₃O-PMP, 5-CH₃O-PMP, 6-CH₃O-PMP appear at 504 nm, 503 nm, 517 nm respectively. Fluorescence spectra of Al(III) complex compound Al(III)-4-CH₃O-PMP, Al(III)-5-CH₃O-PMP, Al (III)-6-CH₃O-PMP show emission peaks at 499 nm, 498 nm and 518 nm, respectively. The fluorescence intensity increased when Al(III) is complexed. Especially, the fluorescence intensity of Al(III)-5-CH₃O-PMP(2B) complex compound is larger than the corresponding ligand by more than twice and could provide a parameter for quantitative analysis of Al(III) ion. The fluorescence intensities for Al(III) complexes are in the following order: Al(III)-5-CH₃O-PMP > Al(III)-4-CH₃O-PMP > Al(III)-6-CH₃O-PMP. This order agrees with the emission results of Al(III) complex with Schiff base 2,2-dihydroxyazomethines.

**The Effect of pH and Ethyl Alcohol.** Fig. 2 shows the fluorescence intensity change of Al(III)-5-CH₃O-PMP complex according to the range of pH. The fluorescence intensity of ligand was subtracted. It is observed that the fluorescence intensity is maximum at pH 5.5–6.2 range. The intensity drops to the minimum value at pH 8.3. It shows another small peak at pH 8.5–9.7 range. It is interesting to note that the maximum fluorescence intensity is observed in weak acid condition rather than in basic condition. This is a great advantage to other ligands in which the highest fluorescence intensity is usually observed in basic condition.

In general, the proton dissociation of phenol OH of the Schiff base ligand occurs in strong base. However, the dissociation of phenol OH proton of 5-OCH₃-PMP occurs in weak acid and forms a complex with Al(III) ion. Thus, selectivity to Al(III) ion becomes higher. If the sample solution is a strong base, protons of all phenols OH dissociate and become anion. There is no competitive reaction between Al(III) ion and protons, and the formation of complex with other metal ions becomes much easier. As a result, selectivity decreases.

In order to find out the most adequate alcohol concentration in the analysis of Al(III) ion concentration of the sample, the volume ratio of ethanol to water was investigated. Below 70% of ethanol content, fluorescence intensity was low, but the intensity increased for 85% of ethanol solution. It seems that fluorescence intensity of Al(III)-5-CH₃O-PMP becomes lower as the alcohol concentration is lower. This is because the solubility of 5-CH₃O-PMP decreases and hydrolysis can occur easier as the alcohol concentration gets lower. Al (III)-5-CH₃O-PMP complex becomes unstable as the alcohol concentration decreases.

**The Effect of Temperature.** Fig. 3 shows the fluorescence intensity change with time at several temperatures. The intensity is highest at 30 °C and decreased with time. Samples placed in water bath at 30–40 °C show low fluorescence intensity in high temperature. As the temperature decreases to 25 °C, the intensity increases and stays constant for 2 hours.
Fluorometric Quantitative Analysis of Al(III) Ion

The calibration curve showed a linear relationship in the range of 0.27 ~ 27 ng/mL. Al(III) concentration. Correlation factor of calibration curve was 0.9989. The sensitivity of the calibration line was considered only for the sample of more than 2.7 ng/mL of Al(III) ion concentration where the reproducibility is acceptable. Although the peak shape of the fluorescence spectra in Fig. 4 is not Gaussian, the peak area is proportional to the peak height within experimental error in our study.

The Effect of Interfering Ions. The fluorescence intensity of each complex after adding interfering ions with known concentration into 1.0 × 10^-6 M 5-CH3O-PMP and 27 ng/mL Al(III) solution was measured. The interfering limit of each ion was set up as 3% tolerance limit (when Al(III) is 27 ng/mL). The result is shown in Table 1. Be(II) ion exhibited interference at the level 7.5 times higher than Al(III) ion. Unlike In(III) ion, Ga(III) ion of 3A family showed interference at the level 3 times higher than Al(III) ion. F^- ion indicated interference at the level 7.5 times higher than Al(III) ion. Other ions did not show significantly high interference level.

In Table 2, our results were compared with the data from the previously published literature in which Schiff base luminous agents was used. Interfering ions whose concentration ranges up to 10 times higher than Al(III) ion were chosen.

Table 1. Effect of foreign ions on the determination of 27 ng/mL aluminum. The interfering ion experiments were performed by adding various concentrations of metal ion solutions to 1.0×10^-6 M 5-CH3O-PMP at pH 6.2.

<table>
<thead>
<tr>
<th>Foreign ion species</th>
<th>Tolerance level (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4^+</td>
<td>Ag(I)</td>
</tr>
<tr>
<td>150,000</td>
<td>100,000</td>
</tr>
</tbody>
</table>

Table 2. Common Schiff base reagents for the spectrofluorimetric determination of aluminum.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Detection limit (ng/mL)</th>
<th>Solvent</th>
<th>pH</th>
<th>Interferences</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Chloro-2-phenylaminomethyl-phenol</td>
<td>0.0027</td>
<td>EtOH/HH2O</td>
<td>10.5</td>
<td>F-, C2O4^2-, Ga(III), Be(II), EDTA, citrate, Cu(II), Co(II), Ni(II), Bi(III), tartrate, acetate</td>
<td>6</td>
</tr>
<tr>
<td>5-CH3O-PMP</td>
<td>0.027</td>
<td>EtOH/HH2O</td>
<td>6.2</td>
<td>Cu(II), Fe(III)</td>
<td>This work</td>
</tr>
<tr>
<td>2,6-Bis[(O-hydroxy)phenylimino-methyl]-1-hydroxybenzene</td>
<td>0.1</td>
<td>EtOH/HH2O</td>
<td>5.0</td>
<td>CT-, F-, PO4^3-, Fe(III), Ga(III)</td>
<td>8</td>
</tr>
<tr>
<td>N-Salicyldiene-2-amino-3-hydroxyfluorene</td>
<td>0.1</td>
<td>EtOH/HH2O</td>
<td>5.2</td>
<td>Gal(III), In(III), Zr(IV), Pd(II), Th(IV), Bi(III), V(V), Cu(II), Fe(III), Ti(IV)</td>
<td>9</td>
</tr>
<tr>
<td>N,N'-Oxacyl-bis(salicylaldehyde hydrazone)</td>
<td>0.2</td>
<td>DMF-H2O</td>
<td>4.7</td>
<td>Co(II), Ni(II), Pd(II), Cu(II), Zn(II), Fe(III), Mn(II)</td>
<td>10</td>
</tr>
<tr>
<td>3-Hydroxypyridine-2-aldehyde-2-pyridylylhydrazone</td>
<td>0.2</td>
<td>EtOH/HH2O</td>
<td>5.6</td>
<td>Cr(III), Co(II), Cu(II), Ga(III), Fe(III), In(III), Mo(VI), Ni(II), Sc(III)</td>
<td>11</td>
</tr>
<tr>
<td>Salicyldiene-o-aminophenol</td>
<td>0.27</td>
<td>DMF-H2O</td>
<td>5.8</td>
<td>Cr(VI), Co(II), Cu(II), Ga(III), Fe(III), In(III), Mo(VI), Ni(II), Sc(III)</td>
<td>11</td>
</tr>
</tbody>
</table>
Table 3. Determination of Al(III) in water samples from various sources. The water samples were prepared by adding HNO₃ and incubated for more than 1h between 35 °C to 45 °C before fluorescence measurements. Results from the fluorescence spectrometric method are shown, together with the values from standard addition method and atomic absorption spectroscopy (AAS) for the comparison.

<table>
<thead>
<tr>
<th>Water</th>
<th>Amount found* : ng/mL</th>
<th>Fluorescence spectrometric method</th>
<th>Standard addition method (SE:SD)**</th>
<th>AAS***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct calibration (SE:SD)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap water (Gyeongbuk Gunwi)</td>
<td>157 (0.36:0.89)</td>
<td>159 (0.18:0.45)</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Tap water (Gyeongbuk Gyeongsan Geumho)</td>
<td>18 (0.22:0.55)</td>
<td>19 (0.18:0.44)</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Hot spring water (Gyeongcheon Sail Onchun)</td>
<td>18 (0.36:0.89)</td>
<td>17 (0.22:0.54)</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Sea water (The East Sea Gyeongbuk Pohang Songdo)</td>
<td>3,348 (1.43:3.56)</td>
<td>3,395 (0.28:0.70)</td>
<td>3,415</td>
<td></td>
</tr>
<tr>
<td>Sea water (The East Sea Gyeongbuk Pohang Bukbu)</td>
<td>1,215 (0.85:2.13)</td>
<td>1,223 (0.41:1.02)</td>
<td>1,244</td>
<td></td>
</tr>
<tr>
<td>River water (Gyeongbuk Pohang Hyeongsan)</td>
<td>375 (0.63:1.58)</td>
<td>383 (0.22:0.55)</td>
<td>385</td>
<td></td>
</tr>
<tr>
<td>Waste water (Gyeongsan waste water treatment area)</td>
<td>651 (1.06:2.65)</td>
<td>661 (0.33:0.72)</td>
<td>666</td>
<td></td>
</tr>
</tbody>
</table>

* Average of five determinations. ** SE: standard error, SD: standard deviation. *** Data were obtained from Research center for instrumental analysis at Daegu University.

It was found that the detection limit of Al(III) ion is 0.0027 ~ 0.27 ng/mL when Schiff base ligand is used. EtOH : H₂O and DMF : H₂O were used as solvents and pH was in the range of weak acid. Interfering cations such as Fe(III), In(III),Ga(III), Be(II) and Cu(II) and interfering anion such as F⁻ were employed. Many interfering ions in Table 2 were also employed when using Superchrome Garnet Y, Pontachrome Blue Black R, Eriochrom red B, Morin, lumogallion, 8-hydroxyquinoline-5-sulfonic acid.

Al(III) Analysis in Natural Water. A fluorometric method using Schiff base ligand was very satisfactory when applied to the quantitative analysis of Al(III) in the natural water samples. Natural water samples were obtained from various sources such as tap water, river water, spa water, sea water and waste water. Quantitative analysis of Al(III) ion content was performed through AAS, standard addition and calibration curve method.

Table 3 compares the results obtained by calibration curve method with standard addition method (AAS method is taken as standard). Error value of Al(III) quantitative analysis was more than 96.2%. It is almost the same to the error value previously calculated.⁵,⁷,⁹

In conclusion, a novel Schiff base ligand 5-methoxy-2-phenyliminomethylphenol (5-CH₃O-PMP) was synthesized and fluorometric method was developed for the quantitative analysis of Al(III) ion in various water samples up to 0.27 ng/mL.

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References