SIMULTANEOUS DETERMINATION OF GALLIUM AND ALUMINIUM IN BIOLOGICAL SAMPLES BY CONVENTIONAL LUMINESCENCE AND DERIVATIVE SYNCHRONOUS FLUORESCENCE SPECTROMETRY

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Summary—The simultaneous determination of gallium and aluminium by using conventional fluorimetry and derivative synchronous fluorescence spectrometry has been studied. These determinations are based on the formation of fluorescent complexes of gallium and aluminium with salicylaldehyde carbohydrazone (SACH). In the conventional method, two samples are analysed under different analytical conditions, and the results are evaluated by solving a system of two simultaneous equations. In the derivative synchronous method (at pH = 2.6, in an ethanol-water medium containing 72% of ethanol), the following conditions are used: a constant wavelength difference of 20 nm between the monochromator settings, a time-constant of 1.5 set, a scan-speed of 120 nm/min, and a derivative wavelength difference of 10 nm; gallium can be determined in the range 7–38 ng/ml, and aluminium between 6 and 45 ng/ml. The synchronous method shows more advantages, and has been used in the determination of both metal ions in diverse biological samples (animal tissues and human serum) with good results.

In recent years, determination of gallium and aluminium at ng/ml levels in water, biological fluids and other materials has aroused considerable interest, though for different reasons. Analysis for traces of gallium is required for studying the physiological distribution of this element in biological systems; such studies are becoming interesting because some gallium compounds have exhibited antitumour activity, but at certain levels gallium shows appreciable toxicity. Several methods for determination of gallium, involving the use of neutron-activation analysis, atomic-absorption spectrometry, atomic-emission spectrometry and fluorimetry have been described.

On the other hand, although aluminium was considered in the past to be a non-toxic element, in recent years the aetiology of various clinical disorders manifest in patients with renal failure has been attributed to aluminium intoxication. The adverse effects of aluminium were most dramatically demonstrated in patients suffering dialysis dementia. Aluminium toxicity is also associated with osteodistrophy, anaemia, gastrointestinal symptoms and possibly cardio-toxicity, the most important source of aluminium in the patients appears to be the water used for dilution of the dialysis solution. In general, graphite-furnace atomic-absorption spectrometry has been used for the determination of aluminium in waters.

Synchronous fluorimetry has been described as a method of improving the selectivity of conventional luminescence spectrometry by taking full advantage of the ability to vary both the excitation and emission wavelengths during an analysis. In this method, the excitation and emission monochromators are scanned simultaneously and synchronously so that a constant wavelength difference is maintained between them. The use of derivative synchronous fluorescence spectrometry was first applied primarily in the analysis of organic compounds, although it can also be used for inorganic mixtures.

This paper describes the use of conventional fluorimetry and second-derivative synchronous fluorescence spectrometry for the simultaneous determination of gallium and aluminium. This determination is based on the fluorogenic reactions of gallium and aluminium with salicylaldehyde carbohydrazone (SACH). The results obtained show that both methods can be used for the simple, rapid, sensitive and selective determination of both elements in a wide variety of samples.

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EXPERIMENTAL

Apparatus

This work was performed on a Perkin–Elmer fluorescence spectrophotometer, model MPF-43 A, equipped with a xenon lamp, excitation and emission monochromators, R-777 photomultiplier, Perkin–Elmer 023 recorder, and a differential corrected-spectra unit (Perkin–Elmer DSCV-2) between the signal output and the record input (by means of this unit, it is possible to generate second-derivative spectra at different wavelength increments).

A Crison Digit-501 pH-meter was used for the pH measurements (throughout this paper pH is used to denote the pH-meter reading and not the negative logarithm of the actual concentration of hydrogen ions in the solution).

Reagents

Materials of analytical-reagent grade or better were used whenever available, and distilled and demineralized water was used throughout.

Solutions of gallium (0.997 g/l.) and aluminium (0.665 g/l.) were standardized by EDTA titration. Working solutions were prepared by suitable dilution.

A $10^{-3} M$ solution of salicylaldehyde carboxydratone (SACH) in ethanol was used (prepared daily); the reagent was synthesized as previously described\textsuperscript{24} and characterized by elemental analysis and infrared and NMR spectroscopy.

Hydrochloric acid-potassium chloride "buffer" solution of pH 1.9 was prepared by mixing 50 ml of 0.2$M$ potassium chloride and 13.3 ml of 0.2$M$ hydrochloric acid and diluting to 200 ml with demineralized water.

Buffer of pH 2.6 was prepared by mixing 33 ml of 0.2$M$ hydrochloric acid with 50 ml of 0.2$M$ potassium hydrogen phthalate and diluting to 200 ml with demineralized water.

Determination of gallium and aluminium by conventional fluorimetry

An aliquot containing 156–950 ng of gallium and 250–1500 ng of aluminium was placed in a 25-ml standard flask, 7 ml of $1 \times 10^{-3} M$ SACH, 7 ml of ethanol and 4 ml of the pH 1.9 solution were added and the solution was made up to the mark with distilled water. The fluorescence intensity ($I_A$) was measured with an excitation wavelength of 360 nm and an emission wavelength of 450 nm.

A similar aliquot was placed in a 25-ml standard flask, and the same volumes of SACH solution and ethanol as before were added, and 4 ml of pH 2.6 buffer solution. The mixture was diluted to the mark with demineralized water, and the fluorescence intensity ($I_B$) was measured with excitation and emission wavelengths of 360 and 440 nm, respectively.

With the $I_A$ and $I_B$ values two simultaneous equations can be established (as described under Results and Discussion). It is necessary to construct calibration graphs for gallium and aluminium separately under each set of fluorimetric conditions (450 and 440 nm emission wavelengths) and to calculate the four slopes.

Determination of gallium and aluminium by second-derivative synchronous fluorimetry

A sample containing 190–950 ng of gallium and 150–1125 ng of aluminium was placed in a 25-ml standard flask, 10 ml of $1 \times 10^{-3} M$ SACH, 8 ml of ethanol and 5 ml of pH 1.9 solution were added, and the solution was made up to the mark with demineralized water. The second-derivative synchronous fluorescence spectrum was recorded by scanning both monochromators together, with a 20 nm constant difference between their wavelengths, a time-constant of 1.5 sec, a scan-speed of 120 nm/min, and a derivative wavelength interval of 10 nm. The excitation monochromator was scanned from 300 to 500 nm, and the emission monochromator from 320 to 520 nm. Peak-to-trough measurements were made in the range 445–470 nm for Al(II) and 405–425 nm for the sum of Al(II) and Ga(III) (the peak-to-trough distances were measured in arbitrary units on the recorder chart) as shown in Fig. 3. The concentration of gallium was obtained by difference.

The fluorescence intensities of these derivative signals are directly related to the concentration of each ion, the concentration of which is determined from the calibration graphs.

Determination of gallium and aluminium in biological samples

Biological samples (0.01–1 g) were digested with a mixture of concentrated nitric acid (10 ml) and 30% hydrogen peroxide (3 ml) in a reflux apparatus. If the material was incompletely decomposed, more nitric acid and hydrogen peroxide were added and the mixture was reheated until a clear solution was obtained. After digestion, samples were evaporated to small volume, neutralized with sodium hydroxide, and finally diluted with demineralized water.
to volume in a 10–50 ml standard flask. A suitable aliquot of this sample solution was pipetted and the gallium and aluminium were determined by the procedure above.

For gallium and aluminium in blood serum a 3-ml portion of the serum was placed in a 10-ml conical centrifuge tube and, with mixing after each addition, 3 ml of 2M hydrochloric acid and 0.8–1 ml of 40% trichloroacetic acid solution were added. The mixture was stirred vigorously with a glass rod for about 45 sec and then centrifuged for 10–20 min at 3000 rpm. A suitable aliquot of the supernatant fluid was pipetted, and the aluminium and gallium content was determined by the procedure above by the method of standard additions or by use of calibration graphs and the two simultaneous equations.

RESULTS AND DISCUSSION

Gallium forms a colourless complex with SACH; the complex exhibits weak absorption in the same region as the reagent alone, but has an intense blue fluorescence, with maximal emission at 450 nm (excitation at 360 nm) (Fig. 1). The reaction is performed at pH 2.4–3.1, in aqueous ethanol medium (60% v/v ethanol). The detection limit is 0.5 ng/ml and the range of application is 1.5–60 ng/ml.

SACH also reacts instantaneously with aluminium to form a complex which has intense fluorescence, with maximum emission at 440 nm (excitation at 362 nm) (Fig. 1). The reaction is done at pH 3.3–3.8 in aqueous ethanol medium (60% v/v ethanol). The detection limit is 1 ng/ml, and the range of application 3–90 ng/ml.

Simultaneous determination by conventional fluorimetry

The simultaneous determination of Ga(III) and Al(III) with SACH by the classical fluorescence technique can be achieved by measurements of the fluorescence due to both complexes under the optimal experimental conditions for each. Thus, two series of samples (A1, A2) are prepared at pH 2.6, and another two (B1, B2) at pH 3.5. In series A1 and B1 (both without aluminium), the concentration of gallium is varied over the range 1.5–60 ng/ml, and in series A2 and B2 (both without gallium) the concentration of aluminium is varied in the range 3–90 ng/ml. In the four series, 7 ml of 1 × 10⁻³ M SACH 7 ml of ethanol and 4 ml of the appropriate buffer solution are added to each sample, and the solutions are diluted to volume in 25-ml standard flasks. The fluorescence intensities are directly related to the concentration of the corresponding metal ion; calibration graphs are

![Fig. 1. Emission spectra of the gallium (A at pH 2.6, C at pH 3.7) and aluminium (B at pH 2.6, D at pH 3.7) complexes. Concentration of each ion is 30 ng/ml.](image-url)
Table 1. Simultaneous determination of gallium and aluminium by conventional fluorimetry

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ga:Al w/w</th>
<th>Ga found. ng/ml</th>
<th>Al found. ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:1</td>
<td>6.3</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
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<td>6.3</td>
<td>5.8</td>
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</tr>
<tr>
<td>10</td>
<td>4:1</td>
<td>40.0</td>
<td>36.0</td>
</tr>
<tr>
<td>11</td>
<td>5:1</td>
<td>50.0</td>
<td>47.0</td>
</tr>
<tr>
<td>12</td>
<td>6:1</td>
<td>60.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

constructed from the results, and the slopes of the four graphs are calculated. From these results, two simultaneous equations are established; in our work these were:

\[ I_A = 0.897 \text{[Ga(III)]} + 0.610 \text{[Al(III)]} \]

\[ I_B = 0.880 \text{[Ga(III)]} + 0.720 \text{[Al(III)]} \]

\( I_A \) and \( I_B \) being the fluorescence intensities measured at 450 and 440 nm, respectively (\( \lambda_{ex} \) 360 nm in both cases), and [Ga] and [Al] the unknown concentrations. Obviously, these equations, as well as those obtained by use of the synchronous technique, depend on the instrument used and the experimental variables, and must be established in each case, according to the procedure described. The concentrations of gallium and aluminium can be obtained by solving both equations. These equations are valid because the fluorescence intensities of the complexes are additive for the stated ranges.

Results obtained for the determination of gallium and aluminium in samples with Ga:Al ratios from 1/6 to 6 are shown in Table 1. They indicate that the method is satisfactory. The relative standard deviations found were 5.4% for gallium and 4.0% for aluminium.

Simultaneous determination by second-derivative synchronous fluorimetry

Figure 2 shows the individual second-derivative synchronous fluorescence spectra of the gallium and aluminium complexes and their mixtures (the concentrations were 7.5 ng/ml for gallium and 20 ng/ml for aluminium in all cases). The distance \( ab \) between the maximum at 445 nm and the minimum at 470 nm is proportional to the concentration of aluminium and independent of gallium concentration. The distance \( cd \) between the maximum at 400 nm and the minimum at 420 nm is proportional to the

Fig. 2. Second-derivative synchronous fluorescence spectra for the gallium (A) and aluminium (B) complexes and for their mixture (C) at \( \Delta \lambda = 20 \text{ nm} \), \( \Delta \lambda' = 10 \text{ nm} \), time-constant 1.5 sec, scan speed 120 nm/min, and pH 2.6. Concentrations are 7.5 ng/ml for gallium and 20 ng/ml for aluminium.
Determination of gallium and aluminium

As a general example, Fig. 3 shows the second-derivative synchronous fluorescence spectra of a series of mixtures containing different concentrations of gallium and aluminium.

The most important parameter in the simultaneous analysis of mixtures by this technique is the selection of the optimum wavelength difference between the monochromator settings (\(\Delta \lambda\)). For selection of the appropriate value, various second-derivative synchronous fluorescence spectra at different \(\Delta \lambda\) values between 10 and 60 nm were recorded. \(\Delta \lambda = 20\) nm was selected as the optimum value. The optimal bandpass was found to be 10 nm for both slits.

For recording the second-derivative spectra, three derivative wavelength intervals (2, 5 and 10 nm) were employed. The maximum response was obtained with the 10 nm interval.

Time-constants of 0.3, 1.5 and 3.0 sec were used in order to study the effect of the spectrofluorimeter response; a value of 1.5 sec was selected. A scan-speed of 120 nm/min was found to be convenient.

The optimum pH range is slightly different for each ion; however, the response function for the resolution of a gallium–aluminium mixture is maximum at an apparent pH of 2.6. A hydrochloric acid–potassium chloride mixture (pH 1.9) was selected, since the volume of ethanol added in the preparation of the solutions increased the pH by 0.7.

Increasing the ionic strength produced no significant changes in the fluorescence. The response function remained constant when there was more than a 36-fold molar excess of SACH. Therefore, 10 ml of 1 \(\times 10^{-3}\)M SACH in a final volume of 25 ml is sufficient.

The optimum amount of ethanol in the final solution was found to be 60–80% v/v; a medium containing 72% ethanol was selected. In this medium the response remains constant for at least 2 hr. There is no temperature effect in the range 10–50°. All measurements were made at 25°.

Several procedures are available for quantitative evaluation of second-derivative synchronous spectra, and a detailed survey of these procedures, including a description of possible errors, has been given by O’Haver and Green.25 In the present work, for the determination of aluminium the amplitude between the maximum centred at 445 nm and the shoulder at 470 nm was selected because the experimental values obtained in this way are related to the aluminium concentration more linearly than those obtained from other possible constructions. The calibration graph prepared by plot-

Fig. 3. Second-derivative fluorescence spectra of gallium and aluminium. (1) Ga, 10 ng/ml; Al, 10 ng/ml. (2) Ga, 20 ng/ml; Al, 25 ng/ml. (3) Ga, 5 ng/ml; Al, 30 ng/ml. (4) Ga, 20 ng/ml; Al, 30 ng/ml.
Table 2. Determination of aluminium and gallium in synthetic mixtures

<table>
<thead>
<tr>
<th>Ga taken, ng/ml</th>
<th>Al taken, ng/ml</th>
<th>Distance ab, mm</th>
<th>Distance cd, mm</th>
<th>Ga found, ng/ml</th>
<th>Al found, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>25</td>
<td>13</td>
<td>55</td>
<td>4</td>
<td>25</td>
</tr>
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<td>5</td>
<td>6</td>
<td>72</td>
<td>38</td>
<td>7</td>
</tr>
</tbody>
</table>

The analysis of samples containing various concentrations of the two ions is feasible over the concentration ranges 7–38 ng/ml Ga(III) (relative standard deviation 2.8%) and 6–45 ng/ml Al(III) (relative standard deviation 3.5%). The detection limit is 1 ng/ml for gallium and 2 ng/ml for aluminium.

Some results obtained for analysis of various mixtures are shown in Table 2.

Interferences

The selectivity of the derivative synchronous method was investigated by determination of gallium and aluminium at the 20 ng/ml level in the presence of various amounts of other ions. The tolerance limits, defined as the concentration causing a deviation of less than ±5% in the analytical response, are listed in Table 3. The tolerance level for certain metal ions can be increased by addition of iodide, citrate, thiosulphate and ascorbic acid.

Applications

To evaluate its effectiveness, the recommended synchronous derivative procedure for the simultaneous determination of gallium and aluminium was applied to a variety of samples, including aquatic plants, aquatic moss, olive leaves, human blood serum, bovine liver, kidney and brain.

In the analysis of the biological samples (except blood serum), a preliminary step is the destruction of the organic matter. Of the various methods tested, the most adequate is the proce-
Table 4. Determination of gallium and aluminium in biological samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ga, ng/ml Added</th>
<th>Found</th>
<th>Al, ng/ml Added</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquatic plant</td>
<td>10.4</td>
<td>9.4</td>
<td>—</td>
<td>42.0</td>
</tr>
<tr>
<td>Aquatic moss</td>
<td>20.0</td>
<td>19.0</td>
<td>10.0</td>
<td>51.0</td>
</tr>
<tr>
<td>Olive leaves</td>
<td>8.0</td>
<td>8.0</td>
<td>15.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Blood serum 1</td>
<td>5.0</td>
<td>5.4</td>
<td>—</td>
<td>5.0</td>
</tr>
<tr>
<td>Blood serum 2</td>
<td>5.0</td>
<td>5.0</td>
<td>—</td>
<td>5.0</td>
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<tr>
<td>Blood serum 3</td>
<td>5.0</td>
<td>5.0</td>
<td>—</td>
<td>5.0</td>
</tr>
<tr>
<td>Blood serum 4</td>
<td>10.0</td>
<td>10.0</td>
<td>—</td>
<td>10.0</td>
</tr>
<tr>
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<tr>
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<tr>
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<tr>
<td>Bovine liver</td>
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<td>11.0</td>
<td>—</td>
<td>12.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>10.0</td>
<td>9.5</td>
<td>15.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Brains</td>
<td>15.0</td>
<td>11.0</td>
<td>—</td>
<td>18.0</td>
</tr>
</tbody>
</table>

Determination described by Bajo et al., in which a mixture of concentrated nitric acid and hydrogen peroxide is used. Blood serum can be analysed without mineralization; protein is removed by means of trichloroacetic acid and centrifugation; gallium and aluminium are measured in the supernatant liquid.

Results obtained in these analyses are given in Table 4.

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REFERENCES