Determination of Aluminum by Fluorometry

1. **Purpose**

This procedure will determine the number of micrograms of aluminum in an unknown by complexing it with a fluorescing agent, extracting into an organic phase and measuring its concentration as a function of fluorescence employing the standard curve quantitation method.

2. **Background**

Fluorescence is the molecular absorption of light energy at one wavelength and its nearly instantaneous emission at another, usually longer, wavelength. Some molecules inherently fluoresce and others must be modified to produce fluorescent compounds. Fluorescent compounds have two characteristic spectra: an excitation spectrum (the wavelength and amount of light absorbed) and an emission spectrum (the wavelength and amount of light emitted). No two compounds have identical fluorescence profiles. It is this principle that makes fluorometry a highly specific analytical technique.

The analytical technique of fluorometry is the measurement of fluorescence. The instrument used to measure fluorescence is called a fluorometer. A fluorometer generates the wavelength of light required to electronically excite the analyte of interest; it selectively transmits the wavelength of light emitted, then it measures the intensity of the emitted light. The emitted light is proportional to the concentration of the analyte being measured. Fluorometers employ monochromators (a spectrofluorometer), optical filters (a filter fluorometer), or narrow band light sources such as light-emitting diodes (LED’s) or lasers to select excitation and emission wavelengths.

One of the most attractive advantages of fluorometry is its inherent sensitivity: fluorometry is 1 to 3 orders of magnitude more sensitive than absorption spectrophotometry. Another advantage of fluorometry over absorption methods is its large linear range.

Fluorometry is a widely accepted and powerful technique that is used for a variety of environmental, industrial, and biotechnology applications. It is a valuable analytical tool for both quantitative and qualitative analysis.

In this laboratory, you will be given an aqueous sample containing aluminum ion. The aluminum ion will be complexed with 8-quinolinol (8-hydroxyquinoline). The reaction is shown in Scheme 1; and it is the rigid 6-membered ring complex that is highly fluorescent. Since the aluminum-quinolinol complex is not soluble in water, this will be extracted into chloroform solution. You will prepare a series of solutions (both standards and unknown) containing the aluminum-quinolinol complex in chloroform solution. Once the chloroform solutions are made, you will determine the fluorescence excitation wavelength and emission wavelength. Using these wavelengths, you will measure the fluorescence intensity of each standard to generate an external calibration curve. You will
also measure the fluorescence intensity of the two unknown solutions to determine the average mass of aluminum present in a 10.00 mL aliquot of the diluted unknown solutions.

\[ \text{Scheme 1. Complexation of aluminum with 8-hydroxyquinoline} \]

3. Materials and Equipment

- Aluminum potassium sulfate
- Assorted glassware
- Chloroform
- Fluorometric buffer
- 8-quinolinol
- Glass fluorometry cuvettes
- Varian Cary Eclipse Fluorometer

4. Safety

Protective eyewear must be worn at all times. Chloroform liquid and vapor is highly toxic and carcinogenic. Whenever handling the chloroform, a pair of blue or purple disposable Nitrile gloves (not beige latex ones) should be worn at all times. All the chloroform procedures should be performed in the fume hood. Dispose of all hazardous waste as directed in this procedure. For more general safety information in the laboratory, please refer to the appendix.

5. Experimental Method

Preparation of standard solution

Accurately weigh out sufficient KAl(SO₄)₂•12H₂O on a small weighing boat to give approximately 25 milligrams of aluminum. Quantitatively transfer with a wash bottle to a half liter volumetric flask. Dissolve, dilute to the mark and mix. Transfer to a plastic bottle and label it "aluminum stock standard solution".
Preparation of dilute standard solutions

The stock standard is too concentrated for the fluorometer. Quantitatively dilute to a concentration of about 1 ppm Al, transfer to a plastic bottle and label it "aluminum working standard #1". Another dilution must be made to approximately 0.1 ppm Al. Quantitatively dilute working standard #1 by a factor of ten and transfer to a plastic bottle. Label this "aluminum working standard #2". Calculate the concentrations of the standard solutions and record in your notebook.

Preparation of the unknown

Obtain an unknown and quantitatively transfer to a 100 mL volumetric flask with nanopure water. Dilute, mix and transfer to a plastic bottle. Label it "Fluorometry unknown".

Complex formation and extraction

NOTICE: FROM THIS POINT ON, YOU MUST COMPLETE THE ANALYSIS IN ONE LAB PERIOD. IT WILL TAKE MOST OF THE LAB PERIOD.

In the hood at the far end of the room will be two stands with six separatory funnels numbered 1 to 6. Rinse these with nanopure water making certain to rinse the stopcocks and stoppers as well. These funnels do not have to be dry.

Add the following to each numbered separatory funnel in the following order. Mix solutions 1 and 2 well.

1. 50 mL nanopure water
2. 2 mL fluometric buffer (from the cupboard)
3. 2 mL 8-quinolinol (from the cupboard)

Swirl the funnels without splashing, to mix the solutions. Since none of above contains your analyte, it is NOT important to know exactly how much of each you have added to the separatory funnel.

Note: the 8-quinolinol solution and the buffer solution are relatively time consuming to make. Please do not waste any of them, if possible. Specifically, there should be NO REASON to be pouring them into a secondary container; use a disposable pipette to measure them out directly from the bottle.

Next, aliquot the following into each separatory funnel:

<table>
<thead>
<tr>
<th>Funnel</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.00 mL working standard #1</td>
</tr>
<tr>
<td>2</td>
<td>5.00 mL working standard #1</td>
</tr>
<tr>
<td>3</td>
<td>10.00 mL working standard #2</td>
</tr>
<tr>
<td>4</td>
<td>10.00 mL unknown</td>
</tr>
<tr>
<td>5</td>
<td>10.00 mL unknown</td>
</tr>
<tr>
<td>6</td>
<td>0 (this is a blank)</td>
</tr>
</tbody>
</table>
Obtain a pair of disposable Nitrile gloves. Rinse the six 50 mL numbered *volumetric flasks* with chloroform by filling Flask 1 about 1/4 full with clean chloroform, stoppering, shaking and pouring into Flask 2, shaking and pouring into Flask 3 etc, etc until all flasks are rinsed with the same chloroform and the last flask (#6) emptied into the large “chloroform hazardous waste” bottle. **Do not rinse the volumetric flasks with water!**

**Caution:** ALL CHLOROFORM PROCEDURES ARE DONE IN THE HOOD. LIQUID AND VAPOR ARE EXTREMELY TOXIC AND CARCINOGENIC!

Spread a large piece of paper towel in the hood on which to set all stoppers. Place the numbered volumetric flasks in front of each corresponding separatory funnel. Set the flask stoppers on the clean paper in front of each numbered flask. Place a small *short-stem funnel* in volumetric Flask #1 and adjust the separatory funnel so the stem of separatory Funnel #1 is in the cone of the short-stem funnel.

Using a graduated cylinder, add approximately 20 mL of clean chloroform to each separatory funnel. For each funnel perform the following steps:

(1) Hold the stopper (to keep from falling out) and vigorously shake it sideways. Invert the funnel (with the stopcock pointing up) and turn the stopcock one complete turn to release any built up pressure.

(2) Set the funnel back in the stand. The chloroform will form a bottom layer containing the aluminum complex.

(3) Remove the stopper and place it on the clean paper. Carefully open the stopcock and allow the chloroform layer to drain into its corresponding volumetric flask. Do not let the solution drain too fast or it will splash out. Drain until the chloroform meniscus almost reaches the stopcock plug then close the stopcock. Don't allow the meniscus to reach the plug or some water will also enter the flask.

(4) Leave the short stem funnel in the flask and remove the flask from under the separatory funnel.

(5) The short-stem funnel will still be wet with chloroform layer and must be rinsed into the flask with some clean chloroform before it can be placed into the next flask. Pour some clean chloroform into a small clean and dry beaker and, using a clean disposable glass (not plastic) pipette, rinse the short stem funnel with a minimal amount so as not to touch the tip of the pipette to the funnel. Also rinse off the stem of the funnel into the flask as you withdraw it.

(6) Place the funnel into the next volumetric flask and repeat this process for all six funnels.
NOTE: If you have not performed a liquid-liquid extraction before, or need a refresher, ask your instructor to go over the procedure with you. It is important to correctly perform the extraction to obtain accurate results. For the extraction it is important to treat all funnels identically to ensure complete mixing and formation of the complex.

Repeat the extraction with a further 20 mL of clean chloroform for all six separatory funnels and drain into the corresponding flasks. Mix and drain the bottom layers into the same flasks as the first time. Using the clean glass pipette, bring the level of each flask to the mark with chloroform. Stopper and mix by inversion.

**Using the Cary Eclipse Fluorometer**

From the cupboard, obtain the rack containing the fluorometry cuvettes. These cuvettes have four transparent sides and must be handled by the tops. In the hood fill the numbered cuvettes nearly full from each corresponding flask. Cap the cuvettes and wipe all sides with a Kim wipe. Take the cuvettes in the rack to the instrument room.

Turn on the instrument and the computer, if it has not been turned on already. (Figure 1, the symbol 0 is off and 1 is on).

![Figure 1: Varian Cary Eclipse Fluorometer System](fluorometry_2.7.doc 8/29/2016 5)
Once the computer has booted up, double-click the "Advanced Reads" icon on the desktop (Figure 2) and wait until the "Advanced Reads" application starts up (Figure 3).

Slide open the sample compartment cover on the left side of the fluorometer and place cuvette #1 in the fluorometer. (Figures 4 and 5)

Click the “Prescan…” button on Advanced Reads. The prescan dialog box will appear. Do not check any of the boxes in this window (Figure 6). Simply click “OK” and the Prescan should start.
Once the prescan has completed, the prescan results should be presented on the computer screen (Figure 7). Record the wavelength values for both excitation and emission in your notebook and click “OK.”

Click the “Create Report” button. Enter your name under the Name field at the top of the window and click “OK” (Figure 9). Click “Print” to print your prescan results. At this time record the PMT voltage (read from the printout) in your notebook.
Now click the “Setup…” button. The setup dialog box should appear (Figure 10). Check the setup dialog box under Cary tab for the following items. If they are not set as shown below, make the appropriate changes:

**Instrument Setup:** Fluorescence  
**Wavelength Setup:**  
- Ex. Wavelength: *(Value from the Prescan result)*  
- Em. Wavelength: *(Value from the Prescan result)*  
- Ex. Slit: 5 nm  
- Em. Slit: 5 nm  
**Average time:** 1 second

If the settings are not as specified above, make the appropriate change(s) and click “OK”.

Place the blank solution in the cuvette holder and click “Zero” button. You will be prompted to load the blank solution in the fluorometer. Click “OK” to zero the instrument (Figure 11).

Once the instrument has been zeroed with your blank solution, click the “Start” button. You should get a warning stating your report will be deleted. Click "OK" (Figure 12).

Next, you will be prompted to the sample selection dialog box. Click "OK" (Figure 13). Finally, you will be prompted to present sample 1. Place cuvette #1 in the sample holder and click "OK" (Figure 14).

Once sample 1 has been read, the instrument will ask for sample 2. Follow the procedure above for the remainder of your samples including your unknowns. Once all the samples are read, click “Print” to print your results. Set up a table and record the emission values in your notebook.
Exit the Advanced Reads application and turn off the instrument.

Dispose all chloroform layers from the flasks and cuvettes into the large “Chloroform Waste” bottle. Pour the contents of the separatory funnels into a separate container labeled “Recovered aqueous solution”. Return the cuvettes to the cupboard. Any unused aqueous solution (excess buffer, 8-quinolinol, or aluminum solution) can be discarded into the sink.

Rinse the separatory funnels with nanopure water and rinse the volumetric flasks with methanol. The methanol waste may be disposed of in the “Recovered aqueous solution” container.

6. Data Analysis/Calculations

Once all the fluorescence emission readings for all five solutions have been made, use the least squares program on Excel to determine the amount of aluminum in each of the 10.00 mL unknown aliquots. Calculate the total number of micrograms of standard aluminum in of the three standard flasks and enter these and your corresponding emission values into the least squares program. Run a least squares analysis for EACH unknown flask. Print out the two results. Remember to add a title to your graph and to properly label each axis. In the conclusion section of your notebook, report your unknown number, each of the individual results and the average number of micrograms of aluminum (to two decimals places) in the 10.00 mL aliquots of unknown.
Instrumental Analysis Laboratory Safety Rules

A. **Instructions**: Carry out all manipulations in accordance with instructions and the safety rules and procedures given herein.

B. **Eye Protection**: All students and staff working in the laboratory must wear **safety glasses at all times**.
   If a student needs to be reminded more than three times to wear goggles, she/he will be dismissed from lab for the remainder of the day, and will not be given an opportunity to make up the work.

C. **Apparel**: The clothes you wear in lab are an important part of your “safety equipment,” and should offer protection from splashes/spills. Closed toed shoes (sneakers are fine), Full-length pants or a full-length skirt, and A shirt that completely covers your torso (i.e. at minimum, a t-shirt).
   In other words, you must NOT wear shorts to lab. You must NOT wear flip-flops, sandals, or crocs. You must NOT wear tank tops, halter tops, spaghetti-strap tops, or low cut jeans to lab. Exposed abdomens, hips, and backs are not safe in the lab.

D. **Gloves**: Gloves are an important part of personal protection. Gloves will be available at all times in the laboratory. Your instructor will require their use when appropriate.

E. **Food**: Food, drinks, and gum are not allowed in lab. None at all, not even water bottles.

F. **Sanitation Issues**: Be sure to **wash your hands** before leaving lab, before you eat anything outside of lab, and before you answer your cell phone.

G. **Music**: Individual headphones are not allowed. Your may choose to play music for the entire class.

H. **Cell Phones and Other Electronic Devices**: Cellular phones and other electronic devices that you do not need to perform your laboratory work should be put away.

I. **Other**: All students are explicitly **prohibited** from:
   1. conducting any unauthorized experiments.
   2. removing chemicals or apparatus from the laboratory for any reason.
   3. working in the lab alone, or at other than regularly scheduled lab periods.
   4. smoking in the laboratory or within 20 feet of any doorway.
   5. impeding movement in aisles or through doorways with bags, skateboards, etc.