ICP-OES DETERMINATION OF IRON.

Introduction:

Silver impregnated cloth has bene used in bandages and dressings to help kill bacteria associated with a wound. Cloth samples are to anaysed for silver and iron to determine if the iron or blood interfered with the silver in the cloth or the cloth was defective and didn't contain the manufacture level of silver. Cloth untainted with blood will be analysed to determine if the cloth originally had silver impregnated. Heme extracted from hemoglobin will be used to stain control cloth to mimic the effect of blood from a wound.

Hemoglobin is a tetramer composed of 4 globin molecules; 2 alpha globins and 2 beta globins. The alpha globin chain is composed of 141 amino acids and the beta globin chain of 146 amino acids. Each globin chain also contains one heme molecule, which is composed of a porphyrin ring containing 4 pyrrole molecules cyclically linked together, and an iron ion ligand bound in the center. For hemoglobin of bovine source, the subunit masses of the protein are about 15,053 and 15,954 Da for alpha- and beta-globin, respectively. The heme group accounts for an additional 616 Da for each subunit.

From a precisely known weighing of intact hemoglobin, the heme groups will be removed by an extraction procedure. The red solution containing iron will be used to stain the silver impregnated cloth. It is of interest in experiment to determine if the heme washes out any of the Ag from the cloth or changes the concentration.

Pre-lab questions:

1) Could mineral content be used as a methodology in fraud or crime scene. Give an example.
2) When selecting emission what parameters are you looking for?
3) Can all ions on periodic table be measured by ICP? Give examples of some that cannot be analysed a determine why.
4) Why should your solvent in standards be similar to sample? Give practical reasons.

Lab 1:

Hemoglobin (25 mg) is weighed and mixed with 3 ml. of RO water and made up to 10 ml. with 0.5% HCl/acetone. The protein chains should precipitate out when the sample is cooled in an ice bath. The heme group remains in the HCl/acetone (top) layer (solution should be red). The protein is in the bottom layer. Centrifuge or filter the solution and keep the supernatant (top layer) and save this material. . Use 0.2 ml of this heme solution to stain the silver impregnated cloth.

Weigh 4 small squares of Ag impregnated cloth samples of approximately 50 mg. into digestion plastic containers. Digest in 1.0 ml. of concentrated nitric acid acid, heat to 90^{o}C,with caps loose then let cool. The solution should be fairly clear. If not digest longer.

Weigh out 4 pieces of the silver impregnated cloth of about 50 mg. each. Stain them in the heme solution and dry them. Digest them as above. Keep all the digested samples for the second lab in the fridge labelled with your group etc.

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Lab: -2.

Make up standard solutions of iron and silver from 1000 ppm stock. The standard for Ag should be in the range of 100 to 1 ppm (5% nitric acid as diluting solution). The standard for Fe should be in the range 5 to 0.05 ppm. Therefore to make standard start with a concentration of 100 ppm Ag, and 5 ppm Fe and do a serial dilution.Each standard final volume should be 5 ml.

Optimize the Varian ICP-OES with the 10 ppm Fe and Ag (about 25 ml) for power, viewing height and nebulizer flow rate using good (see procedure for selecting wavelengths below) Fe and Ag wavelengths. The optimum conditions will be used for measuring the low concentration of iron and silver in the cloth. Optimization is performed using the instrument automatic optimization program “automac”.

Use the optimized settings for power, nebulizer flow, and viewing height.

Analyse the samples prepared and digested from the previous week. The samples should be diluted in Milli Q water so the final volume is 6 ml. This dilution should be good for the analysis of Fe. Redilute 0.1 ml from the 6 ml, 1 to 100 (0.1 to 10 ml). {Samples and/standards may have to be adjusted to cover the same range}.

Instrument setup

Turn on the argon tank, setting the low pressure gauge to 80 p.s.i. The system will begin its purge. This takes about 20 -30 minutes.

Locate the tube holder at the top of the peristaltic pump. Select either pump tubing, and place the bottom and top tab between the bottom and top holders stretching the tubing clockwise over the pump rollers. Repeat for the other tube. Make sure you know what your sample and waste tubes are. T*hey are of different sizes?* **Note that the pump will rotate clockwise, so make sure the tubing is configured in the way so that the sampling tubing will pump the sample into the nebulizer and out from the spray chamber into to the waste container.**

Lock the pressure bars into place by raising the pressure bar onto the tubing and clamp them to their original position. The middle groove of the 3-channel peristaltic pump is reserved for an internal standard or ionization buffer solution.

Place the tubing on autosampler pump and clamp pressure bar.

When the instrument is **finished purging** with argon, turn on the water cooler, located underneath the lab counter behind the ICP-OES. The peltier cooler (instrument screen) should show a decrease in temperature to about **-35^{0}C**.

Igniting the plasma ***(This is done only 15 minutes before you are ready to actually run samples. Set up methods and make standards and samples before starting plasma. The ICP uses expensive argon when running, so timing is important).***

- To turn on the plasma click the ***Green Plasma*** icon. It will take 10-30 seconds for the plasma to ignite. Watch the plasma compartment to make sure that the plasma has ignited properly.

Method

. Click the ***Worksheet*** icon. Click ***New*** to display the ***Create a New Worksheet*** dialog box.

- Click on the "C" , ICP data, 4590. Type a name (**your group name and year)** for the worksheet. Click ***OK***. The ***Method*** page will then be displayed.

-. Click the ***Edit Method*** icon. You can now select the Fe and Ag elements and their wavelengths that are in your samples and standards that you are going to analyze.

The elements are selected from the Periodic Table. Associated with each element is a list of emission wavelengths, intensity, and order. The emission lines are displayed in order based on the most intense and interference free. When clicking on a wavelength, a graph will appear on the top showing the potential interferences (it is also shown in a table on the right side of the screen).

Choose the wavelength of each element based on:

Intensity (highest signal to noise ratio on the chart to the left side)

Least interferences (chart on the right side)

To select the line(s), simply move the cursor to the line and click on ***OK***.

Setting the Concentrations of the External Standards:

- Open the **Standards** tab which will now open a window that indicates all the elements chosen, change the units to the units that you are measuring (mg/L), and number of standards to the number of values you have made (6). Your range of standard should cover the values of your unknowns.

- Insert the assigned standard concentrations values that you have prepared, stating with least concentrated.

- On the lower part of the screen locate the max % error. This value can be changed to 100% to guarantee the standard curve will be developed.

- Close the method window and when prompted to save ***click yes***.

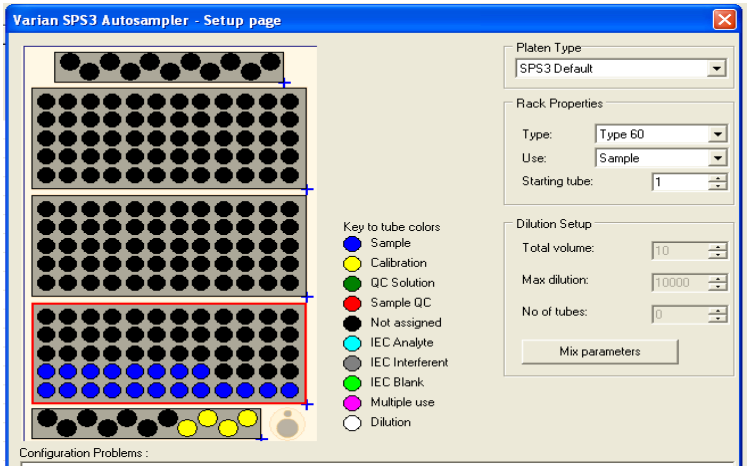
- The multi element method has now been made.

Defining the Sample Sequence

- Click the ***Sequence*** tab to open Worksheet samples table.

- Click the ***Sequence Editor*** tab on the right side of the screen which will open a new page. Type in the sample count. Since you will only analyze a few samples, **uncheck** ***Recalibrate every 10 samples***. Check ***Begin with calibration***, and ***Include a blank in calibration***. Save and close the page.

- On the sequence page, click **Autosampler Setup** tab which will open the Autosampler Setup page. Make sure the Platen Type is SPS 3, Rack Type is Type 60, use is ***Sample***, and ***Starting tube*** is 1. Observe where the calibration standard positions are on the autosampler tray, they are defined by the yellow spots on the screen. The samples are in blue and the 1st spot is the lower left hand corner. Close the page.



- In the Worksheet Samples table, enter in the sample ID. The blank and standards should be shown in the table.

Analysis

- Place the blank (i.e., the wash solution), calibration standards, and samples in the autosampler at the positions defined in the sample sequence table.

- Fit the autosampler pump tubing on the autosampler peristaltic pump. Place the inlet tubing into the wash solution. The outlet is connected to the drain bottle under the lab counter. Make sure the flow are going the right way.

- Under the **Analysis** menu click on **the green play button at the top of the screen**. This will start the analysis, beginning with the calibration blank and standards, and then the samples as defined in the sample sequence.

- Verify the calibration curves for each wavelength. Note that some wavelengths may have failed the calibration due to interference and/or concentration range.

- Complete the analysis of all the samples.

- Inspect the **Analysis Worksheet**. It contains all the analytical results. Pay attention to any flags that may be associated with a concentration. A detailed list of flags can be found from the **Help**

Questions

1. What industries would use an ICP-OES? Give two examples and describe what the ICP would be needed for.
2. What compounds can form in the Argon plasma that might interfere with the analysis?