CHEM 335 INSTRUMENTAL ANALYSIS LAB

Laboratory Manual

Department of Chemistry

İZMİR INSTITUTE OF TECHNOLOGY

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EXPERIMENTS

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Experiment 10. Determination of Methyl Yellow, Methyl Red and Bromocresol Green by TLC and Paper Chromatography

Suggested Readings :

- Skoog D.A.; Holler F.J.; Nieman T.A. ***Principles of Instrumental Analysis - 6th Edn.; Thomson Brooks/Cole: Belmont, CA, USA, 1998.***

Experiment 1. Determination of MnO_4^- and Nickel in Their Mixture by UV-Vis Spectrometry

Topics to be covered (page: 335-398, ch. 13,14)

- Electromagnetic spectrum
- Beer's Law
- Limitations of Beer's Law
- Absorbing species and electronic transitions
- Instrumentation (components and types of instruments)
- Determination of multi-component mixtures
- Applications of UV-Vis spectrophotometer

Purpose:

In this experiment, the mixture of MnO_4^- and Nickel solutions will be analyzed and determined by UV-Vis spectrophotometer.

Introduction:

Absorption of visible and ultraviolet (UV) radiation is associated with excitation of electrons in an atom or molecule to higher energy states. All molecules can undergo electronic excitation following absorption of light. Because absorption spectra are characteristic of molecular structure, they can be used to qualitatively identify atomic and molecular species. The amount of light, I, transmitted through a solution of an absorbing chemical in a transparent solvent can be related to its concentration by Beers Law:

$$-\log\frac{I}{I_0} = A = \varepsilon_\lambda bc$$

where I_0 is the incident light intensity, A is the absorbance (a defined quantity, also referred to as the optical density, or OD), b is the cell path length in cm, c is the solution concentration in moles/liter, and ε_λ is the molar absorptivity, (also referred to as the molar extinction coefficient)

which has units ofliter/mole/cm (i.e., A is a unitless quantity). Notice that ε_λ is a function of wavelength, and it is the quantity which represents the spectrum of the solution. When its value is stated, it must be stated for a particular wavelength (e.g. ε_{532}). Thus absorption spectroscopy can be used to quantify the amount of chemical present in an unknown solution.

Instrumentation:

Instruments for measuring the absorption of UV or visible radiation are made up of the following components;

1. Sources (UV and visible)

2. Wavelength selector (monochromator)

3. Sample containers

4. Detector

5. Signal processor and readout

Solutions:

Stock Nickel solution. Prepare 100 ml of 0.4 M of stock $NiCl_2.6H_2O$ solution in distilled water.

Stock MnO_4^- solution. Prepare 100 ml of 0.002 M of stock $KMnO_4$ solution in distilled water.

Procedure

1) Prepare blank (H_2O), 0.4 M, 0.3 M, 0.2 M and 0.1 M of $NiCl_2$ and $1x10^{-4}$ M, $2x10^{-4}$ M, $3x10^{-4}$ M and $4x10^{-4}$ M of MnO_4^- standard solutions in 50 ml volumetric flasks. In addition, prepare a mixture of Nickel and MnO_4^- with the concentrations of 2 $x10^{-4}$ M each.

2) Scan 0.4 M of Nickel and $4x10^{-4}$ M of MnO_4^- solutions and the prepared mixture from 250 nm to 750 nm and obtain three spectra.

3) Determine two working wavelengths;

a. A wavelength at which MnO_4^- shows maximum absorbance(λ_1)

b. A wavelength at which Nickel shows maximum absorbance(λ_2)

4) Measure the absorbances of MnO_4^- and Nickel standard solutions at two wavelengths and then draw calibration plots for each wavelength.

5) Determine molar absorptivities of MnO_4^- and Nickel at λ_1 and λ_2 by drawing calibration curves of concentration versus absorbance. You will obtain four calibration curve and four ε values.

6) Obtain your unknown mixture from your TA.

7) Measure absorbances of the unknown mixture at the wavelengths determined in **3**.

8) Calculate concentrations of MnO_4^- and Nickel in the unknown mixture.

Experiment 2. Determination of MnO_4^- and Nickel in their mixture by Colorimetry (Visiblephotometry)

Purpose:

In this experiment, the mixture of MnO_4^- and Nickel solutions will be analyzed and determined by Colorimeter (Visible photometer)

Introduction:

Colorimetry is a technique used to determine the concentration of colored compounds in sample solution at visible spectrum of light (400-700 nm) . The device measures the absorbance of particular wavelengths of light by a specific solution.

Instrumentation:

The visible photometry (colorimetry) is made up of the following components;

1. light source

2. filter (the device that selects the desired wavelenght)

3. cuvette chamber (the transmitted light passes through compartment wherei n the solution containing the colored solution are kept in cuvette, made of glass or disposable plastic)

4. detector (this is a photosensitive element that converts light into electrical signals)

5. Galvanometer (measures electrical signal quantitatively)

Solutions:

Stock Nickel solution. Prepare 100 ml of 0.4 M of stock $NiCl_2.6H_2O$ solution in distilled water.
Stock MnO_4^- solution. Prepare 100 ml of 0.002 M of stock $KMnO_4$ solution in distilled water.

Experiment 3. Spectrofluorometric Determination of Aluminum

Topics to be covered (page: 399-429, ch. 15)

- Theory of fluorescence and phosphorescence
- Emission and excitation spectra
- Instrumentation
- Applications of Fluorimetry

Purpose:

In this experiment, concentration of aluminum in a solution will be determined by fluorescence spectrophotometer. To achieve this, aluminum-morin complex will be formed which has fluorescent property.

Introduction:

Molecular fluorescence spectroscopy is based on the emission of light by molecules, which are excited to emit their characteristic spectra by exposure to UV light of specific wavelengths. The wavelength at which a molecule may be excited to emit is referred to as the excitation wavelength, and the spectrum emitted by the sample the emission spectrum. In molecular fluorescence spectroscopy, the emitted spectrum is monitored at right angles to the exciting radiation. The intensity of emission at a specific emission wavelength is proportional to the concentration of the fluorescent molecule. This relationship forms the basis of quantitative analysis with this technique.

Instrumentation:

Instrumentation used to measure fluorescence involves

- an excitation light source
- a device to select the excitation wavelength
- a sample holder
- a device to select the fluorescence wavelength to be monitored
- a photon detector
- readout device

Chemicals and Preparations of Solutions:

1) Al^{3+} stock solution (1000 ppm): $AlCl_3$ is dissolved in water (use a 100 ml volumetric flask) and pH is adjusted to 2.0 with HCl before reaching 100 ml.

2) Al^{3+} standard solution (10 ppm): 1.0 ml of stock solution is diluted with water in a 100 ml volumetric flask.

3) Morin solution: Morin hydrate is dissolved in ethanol. After filtration, 0.5% (w/w) solution is prepared.

4)Ethanol

5) Acetic acid / Sodium acetate buffer (0.1 M): 0.6 ml of acetic acid and 1.36 g of sodium acetate is dissolved in water and diluted to 100 ml in a volumetric flask.

Procedure

1) Place 0.0, 1.3, 2.5, 3.7, and 5.0 ml of 10 ppm Al^{3+} standard solution into 25 ml volumetric flasks separately.

2) Add 0.5 ml of morin solution, 14 ml of ethanol and 5 ml of buffer solution into the volumetric flasks. Then dilute them to 25 ml with water.

3) Repeat step **2** for the unknown solution. Remember dilution factor.

4) After preparation of solutions, wait for 30 minutes.

5) Obtain emission spectra of the solutions in the wavelength range of 480-600 nm and in excitation wavelength 465 nm.

6) Using maximum absorbance values, plot calibration curve and determine aluminum concentration of the unknown sample.

Experiment 4. Determination of copper in tap waters by Flame Atomic Absorption Spectrometry

Topics to be covered (page numbers: 13-24, 215-249i ch. 1,8,9)

- Basic theory of atomic absorption
- Sample atomization techniques
- Atomic absorption instrumentation
- Sources (Hollow cathode lamp)
- Sensitivity and limit of detection
- Calibration methods
- Standard Addition Method

Purpose:

In this experiment the amount of copper in tap waters will be determined by flame atomic absorption spectrometry.

Introduction:

Atomic absorption spectrometry involves the absorption of radiant energy by neutral atoms in the gaseous state. Elements in the samples are atomized in flame or graphite atomizer, and each element absorbs characteristic wavelength-light discharged from cathode lamp. By measuring the absorbance of the light, quantitative analyses are carried out. The element being determined must be reduced to the elemental state, vaporized, and imposed in the beam of radiation from the source. This process is most frequently accomplished by drawing a solution of the sample, as a fine mist, into a flame. The flame thus serves a function analogous to that of the cell and solution in conventional absorption spectroscopy. The light beam from the source is passed directly through the flame.

Quantitative analysis is easily performed by measuring the radiation absorbed by the sample in the flame. Typically a calibration curve is first prepared by measuring the absorbance of a series of solutions of known concentration. The sample(s) is then measured under same

conditions. The instrument analyzes inorganic elements in the samples at the range of ppm (mg/L) and/or ppb (μg/L) levels. About 65 elements can be determined by atomic absorption with sensitivities down to 0.1 mg/L in same cases.

Instrumentation:

Thermo-Elemental Solaar M atomic absorption spectrometer

Starting the system:

After turning the instrument on, plug in the appropriate hollow cathode lamp. Once initialization is complete, create a new method, using the method wizard. Select "Cookbook" method and press the **Next** button to view each of the parameters. Record these parameters in your lab notebook. Press **Lamp** button to turn the lamp on. The lamp should begin to glow.

Optimization:

Aligning the Lamps:

- Once the method has been loaded, ensure the required lamp position is selected with lamp selection located in the carousel position. Allow the lamp time to warm up (about 15 minutes).
- Ensure nothing is in the optical path, and then press the **Optical Set up** button. When the optical set up is finished, the system status will retun to **ONLINE.** The lamp is now aligned and ready for use.

Aligning the Burner:

- Use a piece of white to locate the light path. Do this by placing the paper level on the top of the burner inside the grid window.
- Place the card halfway along the burner slot. Position the card with the vertical line perpendicular to the slot, and adjust the burner height until the light beam falls within the target area.

Lighting the Flame:

- Open the acetylene cylinder. The pressure should already be set to ~ 9 psi. Open the air compressor. Press the 'Flame on' button and keep it pressed until the flame ignites.
- Watch this through the window. If the flame does not ignite or goes out, wait 5 seconds and then try again.
- If this fails, see your TA. Check the gas regulator settings and readjust if necessary.
- When the flame has stabilized, adjust the flame conditions as described in the next section.

Optimizing the Flame Signal:

- Aspirate the blank and click on the **Autozero** button to perform an Instrument Zero. Aspirate a standard solution that will give an absorbance of at least 0.2.
- Watch the signal bar and adjust the impact bead position by gradually turning the impact bead adjustment screw first clockwise, then counter-clockwise to find the maximum absorbance.

  THE SYSTEM IS NOW OPTIMIZED.

Calibrating the Method:

- Open the **Results** window so that you can see your results as they are measured and the calibration window to see the calibration plot as it is built up from the standard solutions.

Running the analysis:

- Click on the **Analyse** button to start the analysis. As the analysis proceeds, you will be prompted to aspirate the solution required for each stage. Aspirate each solution as you are prompted and click on **OK.** The solution will be measured and the result will appear on the **Results** window.

Shutting Down the System:

- Aspirate pure solvent for 10 minutes.
- Shut off acetylene supply at the gas cylinder.
- Shut off air tank.
- Turn off the flame.
- Wait until the hissing sound stops, then turn off the Instrument.

Reagents:

1. 250 ml 100 mg/L Stock Solution of Copper (made by diluting the appropriate weight of copper sulfate pentahydrate, $CuSO_4.5H_2O$)
2. 65 % HNO_3 solution.

Procedure:

Sample and Standards:

1. To seven labeled 50 mL volumetric flaks, prepare the following solutions from 100 mg/L stock solution of copper:
0 (Blank), 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 mg/L Cu standards (before making dilution add 0.5 ml of 65 % HNO_3 solution to each of the standard.)
2. Obtain your tap water from your TA and prepare standards by standard addition method in 1.0% HNO_3. (+0, +0.5, +1.0, +2.0, +5.0, +10.0 mg/L in 50 mL volumetric flask)

Data Sheet

A) AAS Operating Parameters

Operating conditions for Atomic Absorption Spectrometer	
Name of the instrument parts	
Light source	
Background correction	
Wavelength selector	
Detector type	
Spectral line used for Cu determination	

B) Absorbance Readings

Aqueous Standards		**Standard Addition**	
Standards (mg/L)	**Absorbance**	**Standards (mg/L)**	**Absorbance**
0		**+0**	
0.5		**+0.5**	
1.0		**+1.0**	
2.0		**+2.0**	
5.0		**+5.0**	
10.0		**+10.0**	
20.0			

Calculations:

Plot the calibration curve of Abs vs. concentration for both standard addition and aqueous standards in the graph (using the least-squares method.). Calculate the Cu concentration in tap water sample.

Experiment 5. Determination of Sodium, Potassium and Calcium in Drinking Waters by Flame Photometry

Topics to be Covered

• Basic theory of atomic emission.

• Flame photometry instruments and their operation.

•Sample introduction

Purpose:

In this experiment, three elements commonly found in drinking waters (sodium, potassium and calcium) will be analyzed and determined by flame photometry.

Introduction:

Flame photometry, is a relatively old instrumental analysis method. Its origins date back to Bunsen's flame-color tests for the qualitative identification of select metallic elements. There is no need for light source. Flame serves both as an atomizer and excitation source. This relies on the principle that an alkali metal salt drawn into a non-luminous flame will ionise, absorb energy from the flame and then emit light of a characteristic wavelength as the excited atoms decay to the unexcited ground state. The intensity of emission is proportional to the concentration of the element in the solution.

It is suitable for qualitative and quantitative determination of several cations, especially for metals that are easily excited to higher energy levels at flame temperature. These metals are Li, Na, K, Rb, Cs, Ca, Cu, Sr, and Ba.

Element	Emitted wavelength	Flame color
Sodium	589 nm	Yellow
Potassium	766 nm	Violet
Barium	554 nm	Lime green
Calcium	622 nm	Orange
Lithium	670 nm	Red

Instrumentation:

A flame photometer instrument is extremely simple where the sample in solution is aspirated through an aspirator or nebulizer into the flame which is usually a propane / air fuel or, even, a purified natural gas/air mixture. The sample matrix evaporates followed by atomization of the sample. Atoms present in the high temperature zone of the flame are excited to higher energy levels by absorbing energy from the flame. As excited atoms return to the ground state they emit radiation in definite wavelength depending on the energy level from which each atom drop. This gives rise to a line spectrum. However, in flame photometry a pre-selected filter (depending on the atom in question) is used and it is the intensity of the emission line that is practically measured and is related to the original concentration of the sample in solution.

The detector is usually a phototube or a photomultiphier tube depending on the quality of the instrument. The emitted radiation is isolated by an optical filter and then converted to an electrical signal by the photo detector. The analysis of Na, K, Li, Ba and Ca are typically determined at low temperatures, i.e. 1500-2000°C, therefore suitable mixtures are propane/air, butane/air and natural gas/ air.

Chemicals and Reagents

1. Standard Na, K, and Ca solutions (1000 ppm each).
2. Sample of unknown concentrations of Na, K, and Ca.

Apparatus

Flame photometer equipped with Na, K, and Ca filters.

Procedure

1. Prepare standard Na, K, and Ca solutions that are 1, 5, 10 ,20 ,30 ,40 ,50 ,60, 70, 80, 90, and 100 ppm of each metal ion.

2. Follow instructions for the correct operation of the flame photometer available.

3. Adjust the signal, using the Na filter, to zero using distilled deionized water.

4. Read the signal for the Na set of standards and then that of the unknown sample.

5. If the signal obtained for the sample is out of range, dilute a portion of the sample properly till a signal within the range is obtained.

6. Construct a calibration curve for Na in the sample and report your results in ppm.

7. Repeat steps 3-6 for K and finally for Ca and find the concentration of each species in the sample. Results should also be reported in ppm analyte.

- After finishing measurements, draw calibration curve and discuss the applicability of it to the unknown samples by looking at correlation coefficient and dynamic range. Compare your results with the accepted value of drinking waters.

Data Sheet

A) Intensity Readings of standards and samples

Determination of Sodium		**Determination of Potassium**		**Determination of Calcium**	
Standards & Sample	**Emission**	**Standards & Sample**	**Emission**	**Standards & Sample**	**Emission**
Blank		**Blank**		**Blank**	
10 ppm		**10 ppm**		**10 ppm**	
20 ppm		**20 ppm**		**20 ppm**	
30 ppm		**30 ppm**		**30 ppm**	
40 ppm		**40 ppm**		**40 ppm**	
50 ppm		**50 ppm**		**50 ppm**	
Drinking water		**Drinking water**		**Drinking water**	

B)Determination of sodium, potassium and calcium in drinking waters

Determination of Sodium	
Line Equation (y=mx+n)	
R^2	
C_{Na} in drinking water (ppm)	

Determination of Potassium	
Line Equation (y=mx+n)	
R^2	
C_K in drinking water (ppm)	

Determination of Calcium	
Line Equation (y=mx+n)	
R^2	
C_{Ca} in drinking water (ppm)	

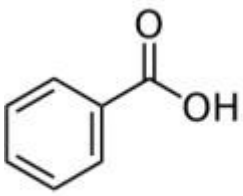
Experiment 6. Infrared Spectrometric Determination of Benzoic Acid and Salicylic Acid

Topics to be covered (page: 430-480, ch. 16, 17)

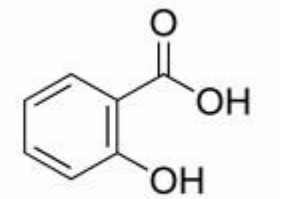
- Molecular vibrations
- Vibrational modes and coupling
- Instrumentation
- Sample handling for solids, liquids, and gases
- Qualitative and quantitative analysis
- Reflection spectrometry

Purpose:

In this experiment, molecular structures of benzoic acid and salicylic acid will be analyzed qualitatively using an infrared spectrometer.



Benzoic acid



Salicylic acid

Introduction:

Infrared absorption is associated with vibrational motions of molecules. Infrared spectroscopy can be used for the determination of organic compounds. Generally infrared spectrum of an organic compound provides a unique fingerprint which is distinguished from all other organic compounds. In this experiment, benzoic acid and salicylic acid are identified.

Spectra of solids that are not soluble in an infrared transparent solvent are obtained by special techniques. Producing pellets by grinding the sample with potassium bromide (KBr) powder is very common to obtain absorbance spectra. As alternatives, diffuse reflectance (DR) and attenuated total reflectance (ATR) accessories are used to obtain spectra without initial sample preparation.

Instrumentation:

An IR instrument contains a source of infrared radiation, a sample container which should be infrared transparent, a wavelength selecting device, a detector and a signal processor, consecutively.

Commercially there are three types of instruments for infrared absorption measurements. These are dispersive instruments, multiplex instruments and non-dispersive instruments.

A dispersive instrument has a monochromator with a grating element to disperse the radiation coming from the source into its wavelengths and it is used as a wavelength selecting device. It is mostly designed double-beam, that is, incoming IR radiation is split into two beams in order to pass through the reference and sample materials.

The most commonly used type of multiplex instruments is Fourier transform (FT) instruments. FT instruments don't have grating element to disperse the light. FT instruments are generally based on the Michelson interferometer. In an FT instrument, IR radiation coming from the source firstly passes through the sample, and then passes through the interferometer.

Non-dispersive instruments are filter or non-dispersive photometers. They are designed for quantitative analysis. Generally they are not complex, easy to use.

Chemicals:

KBr powder
Benzoic acid
Salicylic acid

Procedure

1) Weigh about 100 mg KBr powder, grind in a mortar, make pellet and collect background spectrum.

2) Weigh about 20 mg benzoic acid, mix with about 100 mg KBr powder, grind the mixture in the mortar, make pellet of benzoic acid. Then collect IR spectrum of benzoic acid.

3) Repeat step **2** for salicylic acid.

4) Install DR accessory.

5) Collect background spectrum using mirror disk.

6) Place sufficient benzoic acid into the sample cup and collect IR spectrum of benzoic acid.

7) Repeat step **6** for salicylic acid.

8) Install ATR accessory.

9) Collect background spectrum without placing anything on the sample compartment.

10) Place sufficient benzoic acid on the sample compartment and collect IR spectrum of benzoic acid.

11) Repeat step **10** for salicylic acid.

12) Interpret and compare the spectra and show the functional groups.

Experiment 7. Determination of Fluoride in Toothpaste using Ion Selective Electrode (ISE)

Topics to be covered (page: 659,616, ch. 23)

- Need to determine flouride in toothpaste
- Reference electrode
- Indicator electrode
- Ion selective electrodes
    - Flouride ion selective electrode
- Interferences encountered

Purpose:

In this experiment you will use an ion selective electrode to measure fluoride concentrations in toothpaste.

Introduction:

The fluoride selective electrode produces a potential across a LaF_3, solid ion exchange phase. LaF_3 exhibits affinity toward F^-. With the exception of OH-, it does not interact with other substances.

The fluoride ion electrode contains an internal reference electrode, an internal fluoride standard, and the LaF_3 ion exchange crystal. An external reference electrode must be used to perform the measurement. Assuming that a saturated calomel electrode is used as the external reference, the potentiometric cell may be represented as

$$Ag \mid AgCl,KCl \mid Cl(0.3\ M),\ F(0.001\ M) \mid LaF_3 \mid \textit{test solution} \mid\mid SCE$$

Sources of error include fluoride ion activity changes due to the ionic strength of solution, temperature, which can affect the measured potential through the Nernst equation which governs the electrode potential response and through the several equilibria that fluoride may have with species present in solution, and substances that complex fluoride in solution.

The most important errors are due to competing chemical equilibria. For the active LaF_3 part of the electrode, the most important interfering species is hydroxide, OH^-. The hydroxide ion complexes with LaF_3 crystal itself, in the same fashion as does for F^-. Subsequently, the potential across the crystal will be a function of $[OH^-]$ and will interfere with the fluoride determination. Another pH dependent effect is due to the basicity of F^-. HF is effectively a weak acid and at pH less than 5, acid-base equilibrium will affect the concentration of F- in solution. In order to overcome these problems, solutions needs to be buffered to a pH between 5 and 9 to control hydronium and hydroxide concentrations.

In general, other fluoride chemical equilibria may be active in solution. Fluoride forms complexes with many polyvalent cation species found in natural and processed waters. Si^{+4}, Fe^{+3}, and Al^{+3} all form complexes with fluoride and thus interfere with measurements. The degree of interference depends on the concentration of the complexing ion and that of the fluoride. The addition of a pH 5 buffer containing a strong chelating agent eliminates the pH and polyvalent cation complexation interferences.

Apparatus and Reagents:

1. Corning model 450 digital pH/ion meter, combination pH electrode, fluoride selective electrode, and magnetic mixer with Teflon-coated stirring bar.
2. CDTA (1,2-cyclohexylene-dinitrilo-tetraacetic acid), sodium fluoride, sodium chloride, glacial acetic acid, and sodium hydroxide

Solutions:

1. *5 M Sodium hydroxide*: Dissolve 20 g NaOH in distilled water, cool, and dilute to 100 mL.
2. *100 ppm F stock solution*: Dissolve 0.1105 g NaF in distilled water and dilute to 500 mL in volumetric flask.
3. *10 ppm F standard solution*: Dilute 10 mL 100ppm stock F solution to 100 mL with distilled water.

4. *pH 5.0-5.5 buffer solution*: Add 28.5 mL glacial acetic acid, 29 g NaCl, and 2 g CDTA to approximately 250 mL distilled water in a 500 mL beaker. Stir to dissolve and cool to room temperature. Calibrate the pH meter/combination pH electrode using pH standards. Using the combination pH electrode, adjust the pH of the buffer solution to between 5.0 and 5.5 with 5 M NaOH (about 75 mL will be required). Transfer solution to a 500 mL volumetric flask and dilute to the mark with distilled water.

Calibration:

Calibrate the electrode as follows. Prepare a series of fluoride standards using the 10 ppm F standard solution in the range of 0 to 2.00 mg/L by diluting appropriate volumes to 50.0 mL using the series below:

Volume of standard solution (mL)	**Standards (mg/L)**
0.00	0.00
1.00	0.20
2.00	0.40
3.00	0.60
4.00	0.80
6.00	1.20
8.00	1.60
10.00	2.00

Procedures:

Sample preparation:

Weigh 200 mg of toothpaste in a small weighing boat and transfer quantitatively to a 250 mL beaker and add 25 ml water. Boil the mixture for 2 min., cool, and transfer quantitatively to a 250 ml volumetric flask. Add 1.4625g of solid NaCl and dilute to the mark.

Measurement procedure:

Set up the pF meter by connecting the fluoride selective electrode. Readings can be taken in potential (mVolts). To prepare for measurement, place 15.0 mL of sample or standard and 15.0

mL of buffer solution in a 100 mL beaker. Place beaker on magnetic stirrer and mix at medium speed. Immerse electrodes in the stirred solution. When taking measurements, electrodes must remain in solution for at least 3 minutes and until the potential readings have stopped drifting.

Data Sheet

Standards (mg/L)	**Potential Readings (mV)**
0.00	
0.20	
0.40	
0.60	
0.80	
1.20	
1.60	
2.00	
Sample	

Calculations:

1. Plot potential measurement of fluoride standards against concentration on two-cycle semi-logarithmic graphic paper. Plot milligrams F^- per liter on the logarithmic axis (ordinate), with the lowest concentration at the bottom of the graph. Plot millivolts on the abscissa.

2. Read the corresponding fluoride concentration from the standard curve, from the potential measurement for each sample.

3. Calculate % F in the original toothpaste sample.

Experiment 8. Determination of o-Xylene, p-Xylene and Toluene by GC and GC-MS

Basics of Chromatography

(page numbers:762,787, ch. 26).

• Know how a separation occurs in chromatography

• Be familiar with the terminology of chromatography. Some of the terms you should know are column, chromatogram, stationary phase, mobile phase, elution, eluent, eluate, solute, distribution constant, retention factor, separation factor, efficiency, band broadening, theoretical plate, and resolution

• Know all the different variables that affect the column efficiency

• Know the relationship between column efficiency and resolution

• Be familiar with the advantages and disadvantages of column chromatography

• Be able to explain how qualitative and quantitative information obtained form chromatography

Gas Chromatography

(page numbers:788,815, ch. 27).

• Know the principle of separation in gas chromatography

• Be familiar with the basic instrumentation in gas chromatography and know each

• Know the advantages of capillary columns compared to packed columns

• Be able to select a specific detector and explain when to use that type of detector

• Be able to measure important quantities from a chromatogram (e.g. t_R, t_M, w, $w_{½}$)

Introduction:

Toluene and the xylenes all belong to a group of organic compounds known as alkyl benzenes. The primary source of these alkyl benzenes in the environment is the petroleum industry and, to a lesser extent, the coke industry. Toluene and the xylenes are all used extensively as solvents and as raw materials in the synthesis of a variety of chemicals. All are used to some extent in the rubber and plastics industries, and all have been used as gasoline additives.

The alkyl benzenes are recognized primarily as atmospheric pollutants mainly because of their high volatility, but small amounts may enter aquatic and terrestrial systems (e.g., gasoline spills from leaking storage tanks).

In this experiment you will analyze an unknown sample containing o-xylene, p-xylene and toluene by gas chromatography (GC). It is one of the most popular chromatographic methods commonly used for the separation of non-polar compounds with high vapor pressure and a vaporization without decomposition.

Roughly, a gas chromatograph is composed from the carrier gas supply, the sample inlet, the column positioned in a column oven and the detector. When a sample is injected into the column, the carrier gas transports the vaporized sample through the thermostated capillary column into the detector. As the sample mixture moves through the column, sample components that interact strongly with the stationary phase spend more time in the stationary phase vs. the moving gas phase and thus require more time to move through the column. When a substance leaves the column, it is sensed by a detector and the separated analytes are obtained as peaks in a chromatogram.

Reagents:

1. Xylene mixture (o-xylene + p-xylene)
2. Toluene
3. Dichloromethane
4. Syringe (10 μL)

Solutions:

Preparation of o-xylene, p-xylene and toluene standards
Std 1 : 200μL **o-xylene** in 5 mL dichloromethane.
Std 2 : 200μL **p-xylene** in 5 mL dichloromethane
Std 3 : 200μL **toluene** in 5 mL dichloromethane

Procedure:

1. Set the instrument to optimum operating conditions for the determination of o-xylene, p-xylene and toluene mixture. Then, record all the information given on your data sheet.

2. Rinse the syringe with at least 3 volumes of your solution and dispense the rinses into the waste beaker.

As you take your sample press the needle through the septum of the injector. Inject rapidly and wait for a few seconds again before withdrawing the needle. ***Do not touch the injector head. It is HOT!***

3. Inject 1.0 μL of o-xylene at 80 ^{0}C and 140 ^{0}C respectively and determine its retention time.

4. Inject 1.0 μL of p-xylene at 80 ^{0}C and 140 ^{0}C respectively and determine its retention time.

5. Inject 1.0 μL of toluene at 80 ^{0}C and 140 ^{0}C respectively and determine its retention time.

6. Inject 1.0 μL of o-xylene, p-xylene and toluene mixture into the gas chromatograph under the same conditions used for the standards.

7.Print the chromatogram of o-xylene, p-xylene and toluene standards.

8. Analyze the same mixture at 60 ^{0}C by GC-MS.

Data Sheet

A) GC operating parameters

GC Conditions for the determination of toluene, o-xylene and p-xylene	
Name of the instrument parts	
Carrier gas	
Injector temperature	
Column temperatures	
Detector temperature	
Column type	
Detector type	
Flow rate	
Sample size	

B) Properties of Compounds in Sample

Compound	**Boiling Point**	**Molecular weight**	**Chemical Formula**
Toluene			
o-xylene			
p-xylene			

C) Data for standards at T_1 (attach your chromatograms)

Compound	**Peak #**	**Retention Time**	**Peak Resolution at .....^{0}C**	**Toluene**	**o-xylene**	**p-xylene**
Toluene				**x**		
o-xylene				**x**	**x**	
p-xylene				**x**	**x**	**x**

E) Data for standards at T_2 (attach your chromatograms)

Compound	**Peak #**	**Retention Time**	**Peak Resolution at..... ^{0}C**	**Toluene**	**o-xylene**	**p-xylene**
Toluene				**x**		
o-xylene				**x**	**x**	
p-xylene				**x**	**x**	**x**

Calculations:

Make your calculations on a separate sheet in your lab report based on the instructions given in the Analysis of Data part.

Analysis of Data:

1. Use the toluene, o-xylene and p-xylene standards to identify the each peak in your sample.
2. Record retention time of toluene, o-xylene and p-xylene standards on your data sheet.
3. Identify each peak in the chromatogram obtained.
4. Calculate the peak resolution of each standard relative to each other.
5. Comment on the results according to your data and calculations.
6. Do not forget to draw the block diagram of GC in your lab report and specify each component according to the instrument you have used.

Experiment 9. Determination of Caffeine Content in Normal and Diet Cola Samples by HPLC

Liquid Chromatography

(page numbers: 762,787, 816-828, ch 26,28).

• Be familiar with the different types of liquid chromatography

• Know what column band broadening is and how it can be minimized

• Understand the difference between normal phase and reverse phase partition chromatography

• Know typical mobile and stationary phases, and be able to predict elution order for a series of compounds

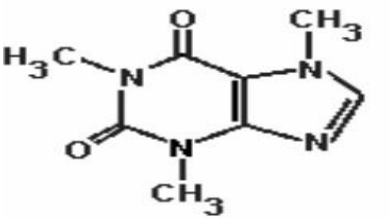
• Be able to explain how eluent strength affects the separation

• Be able to distinguish between gradient and isocratic elution

• Explain the differences between liquid and gas chromatography

Introduction:

Caffeine is a common organic molecule found in many beverages such as coffee, tea, cola and some foods that we consume. Caffeine is a stimulant to the central nervous system. That is why many people drink coffee or soda to help them feel alert.



Caffeine has a mildly addictive effect on the body. When taken in small amounts caffeine is harmless, it is even considered beneficial; although, when taken excessively or in large doses for an extended period of time it has been proven to be detrimental. Therefore, it is interesting to know exactly how much caffeine is present in certain beverages.

This experiment provides an introduction to the application of High Performance Liquid Chromatography (HPLC) since it is the one way of determining the amount of caffeine present in these beverages.

HPLC utilizes a liquid mobile phase to separate the components of a mixture. These ccomponents (or analytes) are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure. In the column, the mixture is resolved into its components.

The main components of an HPLC system are a high-pressure pump, a column and an injector system as well as a detector. The system works as follows: eluent is filtered and pumped through a chromatographic column, the sample is loaded and injected onto the column and the effluent is monitored using a detector and recorded as peaks.

If a series of caffeine standards are analyzed, then the amount of caffeine in another substance can be determined. In this lab, you will prepare standard solutions of known caffeine concentration and find the caffeine content of some common cola samples.

Reagents:

1. Acetic acid (99.8%)
2. Sodium acetate
3. Acetonitrile
4. Caffeine

Caffeine is a common organic molecule found in many beverages such as coffee, tea, cola and some foods that we consume. Caffeine is a stimulant to the central nervous system. That is why many people drink coffee or soda to help them feel alert.

Solutions:

Preparation of mobile phase

1. 10% Acetonitrile: 90% Buffer solution (CH_3COOH-CH3COONa).
2. Prepare a 0.02 M of 1L CH3COONa solution.
3. Add CH_3COOH drop by drop until the pH value is 4.3, then mix 900 mL of buffer solution thus prepared with 100 mL acetonitrile.

Preparation of caffeine standards and samples

1. Prepare 100 ppm of 100 mL caffeine stock solution, then prepare 5 ppm- 10 ppm- 15 ppm- 20 ppm of standard caffeine solutions from 100 ppm stock.
2. Obatin a beverage sample.
3. For cola samples, degas the sample by placing it in an ultrasonic bath. Keep it until no more CO_2 bubbles appear in the sample.

Procedure:

1. Set the instrument to optimum operating conditions for the determination of caffeine. Then, record all the information given on your data sheet.
2. Rinse the syringe with at least 3 volumes of your solution and dispense the rinses into the waste beaker.
3.Inject about 20-25 µL of each standard caffeine solution or sample.
4. Switch the manual injector to the "load" position and push at least three 25-µl volumes of each sample through the sample loop, making certain each volume does not contain air bubbles.
5. With the syringe still in the injector, turn to the "inject" position, at which time the instrument will automatically start data acquisition. Leave the injector in the "inject" position, and remove the syringe.
6. Print the chromatogram of each standard caffeine solution.

Data Sheet

A) HPLC operating parameters

HPLC Conditions for the determination of caffeine in cola samples	
Name of the instrument parts	
Column type	
Detector type	
Mobile phase	
Flow rate	
Loop size	

B)Data for standards (attach your calibration curve)

Standards	Retention Time	Peak Area

B) Caffeine determination in cola samples

Beverage	Retention Time	Peak Area	Concentration	% Error

Calculations:

Make your calculations on a separate sheet in your lab report based on the instructions given in the Analysis of Data part and determine the caffeine content in your samples.

Analysis of Data:

1) Use the caffeine standards to identify the caffeine peak.
2) Record retention time and and peak area for each of the standards on your data sheet.
3) Prepare a *calibration curve* by graphing peak area versus concentration of caffeine in ppm using the data obtained for the standard solutions.
4) Determine the amount of caffeine in cola samples. Report your results as instructed by your TA.
5) Use Table 1 to calculate the percent error between the results obtained in this experiment and the approximate amount of caffeine in your selected beverage.
6) Do not forget to draw the block diagram of HPLC in your lab report and specify each component according to the instrument you have used.

Table 1 – Approximate guide for caffeine amounts in common beverages.[1]

Beverage	Caffeine (mg/mL)
Coca Cola	0.128
Diet Coke	0.128
Pepsi	0.105
Diet Pepsi	0.100
Tea, Iced	0.197
Tea, brewed, imported	0.290
Tea, brewed, domestic	0.193
Tea, instant	0.145
Drip Coffee	0.652
Espresso	1.690–2.25

[1]National Soft Drink Association and Bunker and McWilliams, J. Am. Diet, 74:28-32, 1979.

Experiment 10. Determination of Methyl Yellow, Methyl Red and Bromocresol Green by TLC and Paper Chromatography

Purpose:

Thin layer chromatography (TLC) is a technique widely used in industrial and research chemistry. It is an inexpensive and simple technique, which makes it attractive for undergraduate chemistry lab experiments. In this experiment, Methyl Yellow, Methyl Red and Bromocresol Green compounds will be determined by using TLC and paper chromatography techniques.

Introduction:

Thin layer chromatography (TLC), along with paper chromatography, is commonly used in student experiments as the initial step toward teaching preparative chromatography, as an introduction to chromatography, reaction monitoring, food chemistry, and analysis of plant extracts. In industry, TLC is also used to identify compounds, determine sample purity, and determine colors and other components in food and cosmetics. TLC is also used for bioautograms to determine purification methods for antibiotic natural products, and for flash chromatography method development.

All of these uses benefit greatly from consistent and reproducible TLC. Unfortunately, improper TLC technique is common in terms of using an unsealed developing chamber, and/or allowing insufficient time for solvent vapors to equilibrate. Incorrect techniques can be found in online resources. Using the correct technique improves consistency between students' experiments and greatly reduces questions about differences in results. Correct technique helps to troubleshoot chromatographic problems, such as whether the student followed the instructions regarding preparing the developing solvent. It is also easier to teach chromatography with the concept of weak and strong solvents if the TLC is run well, and the evaluation of different TLC procedures could be made into a lab experiment itself.

Analysis and Discussion:

The Incorrect Method:

A common incorrect method involves spotting a TLC plate, adding development solvent to a beaker, immediately placing the TLC plate in the beaker, and covering the beaker with a watch glass. Although some useful information can be gained, the results are inconsistent with respect to distance moved by the spots, even if run by the same person, as the solvent has no time to equilibrate with the atmosphere inside the beaker. Vapor also escapes from the gap between the watch glass and the beaker spout.

Correct Method:

In the proper method, the developing chamber is sealed, and the solvent has a chance to equilibrate with its vapor. For most organic solvents, equilibration is essentially complete by the time a TLC plate is prepared (3−5 min); solvent systems incorporating water, however, will need several hours to become equilibrated. Using paper (towel or filter paper) facilitates equilibration. Although there are specialized developing chambers, they are often costly, while less expensive alternatives, such as wide-mouth jars, can be used with minor modification. When using beakers, the watch glass can be replaced with aluminum foil with the added advantage that it is one less thing to break.

Method Development:

As to why running a TLC plate properly is important, the example of a flash chromatography method development is used. The retention of a compound on a preparative column under isocratic conditions can be predicted from a properly run TLC plate by eq 1.

$$R_f = \frac{1}{(1+k)}$$

Here, R_f is the retention factor, and k is the capacity factor for the column run under the same solvent system used to develop the TLC plate. The capacity factor is a measure of the retention of a compound on a column described by eq 2.

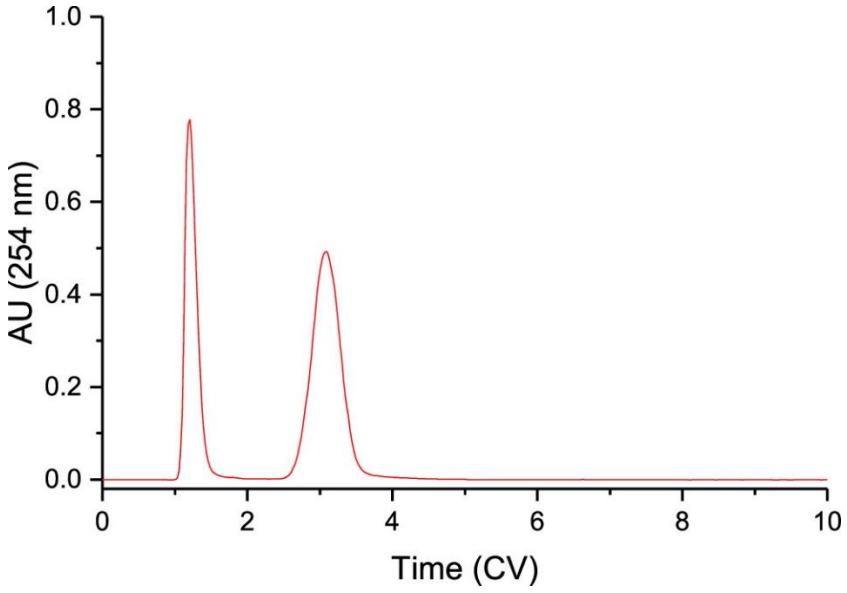
$$k = \frac{(t - t_0)}{t_0}$$

In this equation, t is the time for a compound to elute, and t_0 is the column's void volume,

which is the space in a packed column filled with solvent, also called the “column volume”. Given that CV = 1 + k, some algebra on these equations yields eq 3, which relates the number of column volumes (CV) needed to elute a compound with a given R_f. A compound with an R_f of 0.3 needs approximately 3 CV to elute, if the TLC plate were run properly.

$$CV = \frac{1}{R_f}$$

The chromatogram in Figure was run isocratically using 10% ethyl acetate predicted by a properly run TLC plate. The TLC plate run incorrectly at 5% ethyl acetate predicted an elution time less than 2 CV. As ethyl acetate is the strong solvent in this method, the prediction from this plate would be incorrect, with the compound eluting much later than expected.



Flash chromatogram of dimethyl yellow, run in 10% ethyl acetate in hexanes, elutes in 3 CV as predicted by the TLC plate. The first peak at ~1.2 CV is the toluene used to dissolve the sample.

Other runs are shown in Table. TLC and chromatography runs for methyl yellow in hexane/ethyl acetate are shown for different solvent compositions; the TLC was run correctly and showed the expected retention. Another compound, thymine, was run with dichloromethane (DCM) and methanol (MeOH) run in a proper fashion, and without equilibration of the solvent.

% *B*	Solvent Front (cm)	Spot Movement (cm)	R_f	Calculated Elution (CV)	Actual Elution (CV)
Ethyl Acetate in Hexanes, Dimethyl Yellow					
5	7.3	1.5	0.21	4.9	4.9
10	7.2	2.4	0.33	3.0	3.1
20	7.5	3.5	0.47	2.1	2.2
MeOH in DCM (Thymine)					
10 (properly run)	7.2	1.8	0.25	4.0	4.1
10 (run without solvent vapor equilibration)	7.4	4.6	0.62	1.61	Not run

TLC: Predicted and Actual Elution Times of Dimethyl Yellow and Thymine

In all cases where the TLC plate was run correctly, there was excellent agreement between the calculated and actual elution.

Conversely, running a silica column with a particular solvent composition will yield the R_f of a properly run TLC plate using the same solvent composition. For the first run in Table, the value of t is 4.9, t_0 is 1 CV, which gives a k of $(4.9 - 1)/1 = 3.9$; using eq 1, $Rf = 1/(1 + 3.9) = 0.204$, very close to the measured value of 0.21.

Experimental Section:

Three TLC chambers were prepared with methanol, ethanol and n-propanol respectively. These were allowed to equilibrate while the TLC plates were prepared. A sample of the methyl yellow solution was taken with a 10 μL micropipet, and 1/3 of the contents was spotted onto each of 3 silica TLC plates. The same procedure was applied for methyl red and bromocresol green. The plates were placed, with one in each chamber.

Calculations:

Methyl Yellow	Mobile Phase	Retention factor	Methyl Red	Mobile Phase	Retention factor	Bromocresol Green	Mobile Phase	Retention factor
	methanol			methanol			methanol	
	ethanol			ethanol			ethanol	
	n-propanol			n-propanol			n-propanol	

Determination of Sodium, Potassium, and Calcium in Drinking Waters by ICP- AES (Demo Experiment)

Topics to be Covered (page numbers: 254-280, ch. 10)

- Basic theory of atomic emission.
- ICP instruments and their operation.
- Inductıvely Coupled Plasma Atomıc Emissıon Spectroscopy
  - o Plasma generation
  - o Sample introduction
  - o Axial and radial plasma
- Benefits of a plasma versus a flame source.
- How does an ICP work ?

Purpose:

In this experiment, three elements commonly found in drinking water (sodium, potassium and calcium) will be analyzed and determined. The ICP will be operated in the sequential-multielement mode.

Introduction:

Inductively coupled plasma (ICP) emission spectrometry is a technique that is used primarily for the determination of trace concentrations of metals. The goal of ICP is to get elements to emit characteristic wavelength specific light which can then be measured. In ICP, a conducting gaseous mixture, plasma, containing a significant concentration of cations and electrons is formed.

In the context of spectrometry, plasma can be defined as a partially ionized gas with sufficiently high temperature to atomize, ionize, and excite most elements. Plasma sources, in general, have several benefits over sources used in emission spectroscopy, with the main advantage of being highly energetic atomization sources. ICP is a type of flame that reaches temperatures of (6000-10000 K), much higher than those reached in ordinary combustion flames. Therefore, the atomization in ICP is more complete and the signal is correspondingly enhanced that enables much lower detection limits to be achieved.

Instrumentation:

An Inductively Coupled Plasma system typically includes the following components:
-Sample introduction system (nebulizer)
-ICP torch
-High frequency generator
-Transfer optics and spectrometer
-Computer interface

An ICP requires that the elements which are to be analyzed be in solution. The nebulizer transforms the aqueous solution into an aerosol. The basic set up of an ICP consists of three concentric tubes, most often made of silica. These tubes are termed outer, intermediate and inner loops which collectively make up the torch of the ICP.

The torch is situated within a water cooled coil of a radio frequency (RF) generator. As flowing gases are introduced into the torch, the RF field is activated and the gas in the coil region is made electrically conductive. The sequence of events forms the plasma. In order to prevent possible short-circuiting as well as meltdown, the plasma must be insulated from the rest of the instrument. Insulation is achieved by the concurrent flow of gasses through the system.

Three gases flow through the system - the outer gas, intermediate gas, and inner or carrier gas. The outer gas is typically Argon or Nitrogen. The purpose of the carrier gas is to convey the sample to the plasma. Argon is commonly used for both the intermediate gas and inner or carrier gas.

The light emitted by the atoms of an element in the ICP must be converted to an electrical signal that can be measured quantitatively. This is accomplished by resolving the light into its component radiation (diffraction grating) and then measuring the light intensity with a photomultiplier tube at the specific wavelength for each element line.

Experimental:

Preparation of Standard Solutions:

Main Stock: 50 mL of 50.0 ppm Na, Ca, K standard solution. Take 2.5 mL of 1000 ppm stock solution of the mult ielement into a 50 mL volumetric flask and dilute to exactly 50 ml. Before making dilutions add sufficient HNO_3 solution to the standards to get a final concentration of 1% (v/v) HNO_3.

Std.1 (50ml of 0.5 ppm std): Take 0.5 ml of stock solution into a 50 ml volumetric flask and dilute to exactly 50 ml.

Std.2 (50 ml of 1.0 ppm standard solution): Take 1.0 ml of main stock solution into a 50 ml volumetric flask and dilute to exactly 50 ml.

Std.3 (50 ml of 2.0 ppm standard solution.): Take 2.0 ml of main stock solution into a 50 ml volumetric flask and dilute to exactly 50 ml.

Std.4 (50ml of 5.0 ppm std): Take 5.0 ml of stock solution into a 50 ml volumetric flask and dilute to exactly 50 ml.

Std.5 (50 ml of 10.0 ppm standard solution): Take 10.0 ml of main stock solution into a 50 ml volumetric flask and dilute to exactly 50 ml.

Blank: Prepare a 1% (v/v) HNO_3 solution.

Preparation of Samples:

Sample 1: For the determination of Na^+only, dilute the sample 10 times

Sample 2: For the determination of Ca^{2+} and K^+, use the sample undiluted

Note: Samples are also should be in 1 % (v/v) HNO_3.

Data Sheet

A) ICP-AES Operating Parameters under Optimized Conditions

Operating conditions for ICP-AES			
Name of the instrument parts		**Detector voltage (V)**	
Wavelength selector		**Plasma gas flow rate (L/min)**	
Detector type		**Auxilary gas flow rate (L/min)**	
Spectral lines used for Na		**Integration time (s)**	
Spectral lines used for K		**Pump flow rate (rpm)**	
Spectral lines used for Ca		**RF Power**	

C) Intensity Readings of Na (I) 588.995 nm at different Instrumental conditions

i) Plasma Gas Flow rate and Pump flow rate is constant

Detector voltage, V	Intensity
1.	
2.	
3.	

ii) Detector Voltage and Pump flow rate is constant

Plasma gas flow rate, L/min	Intensity
1.	
2.	
3.	

iii) Detector Voltage and Plasma gas flow rate is constant

Pump flow rate , rpm	Intensity
1.	
2.	
3.	

C) Intensity Readings of standards and samples

Standards & Samples	Intensity Readings					
	Na (I)	Na (I)	Ca (I)	Ca (II)	K (I)	K (I) .....
Blank						
Std. 1						
Std. 2						
Std. 3						
Std. 4						
Std. 5						
Sample 1						
Sample 2						

Standards & Samples	Corrected Intensity Readings					
	Na (I)	Na (I)	Ca (I)	Ca (II)	K (I)	K (I) .....
Blank						
Std. 1						
Std. 2						
Std. 3						
Std. 4						
Std. 5						
Sample 1						
Sample 2						

Calculations & Discussions:

- Calculate the concentration of Na^{+}, Ca^{2+} and K^{+} in the water from some calibration graphs are obtained at the end of the experiment.
- Discuss the effect of detector voltage, pump flow rate and plasma gas flow rate on signal intesity
- Explain why the intensity of different emission wavelength for the same element is different from each other.