# KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore – 641 021.
(For the candidates admitted from 2018 onwards)

## DEPARTMENT OF CHEMISTRY

**SUBJECT NAME: INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS SUBJECT CODE: 16CHU514A**
**SEMESTER: V CLASS: III B.Sc CHEMISTRY**

## INSTRUMENTAL METHODS OFCHEMICAL ANALYSIS- PRACTICAL

**Programme objectives**

The course enables the student to

1. Understand the Safety Practices in the Chemistry Laboratory
2. Understand the testing of chemicals using UV-Visible spectrophotometer
3. Understand the testing of chemicals using IR, GC and NMR spectrophotometer
4. Understand the testing of chemicals using chemical methods

**Programme outcome**

The student knows

1. The Safety Practices in the Chemistry Laboratory
2. The testing of chemicals using UV-Visible spectrophotometer
3. The testing of chemicals using IR, GC and NMR spectrophotometer
4. The testing of chemicals using chemical methods

**Experiments**

1. Safety Practices in the Chemistry Laboratory
2. Determination of the isoelectric pH of a protein.
3. Titration curve of an amino acid.
4. Determination of the void volume of a gel filtration column.
5. Determination of a Mixture of Cobalt and Nickel (UV/Vis spec.)
6. Study of Electronic Transitions in Organic Molecules (i.e., acetone in water)
7. IR Absorption Spectra (Study of Aldehydes and Ketones)
8. Determination of Calcium, Iron, and Copper in Food by Atomic Absorption
9. Quantitative Analysis of Mixtures by Gas Chromatography (i.e., chloroform and carbontetrachloride)
12. Potentiometric Titration of a Chloride-Iodide Mixture
14. Nuclear Magnetic Resonance
15. Use of fluorescence to do ―presumptive tests‖ to identify blood or other body fluids.
16. Use of ―presumptive tests‖ for anthrax or cocaine
17. Collection, preservation, and control of blood evidence being used for DNA testing

20. Laboratory analysis to confirm anthrax or cocaine
22. Detection of illegal drugs or steroids in athletes
23. Detection of pollutants or illegal dumping
24. Fibre analysis

*At least 10 experiments to be performed.*

**Suggested Readings**

**Text Books:**

1. Douglas A. Skoog, James Holler,F. & Stanley Crouch (2007). *Principles of Instrumental Analysis* .6th Edition.(ISBN 0-495-01201-7).

**Reference Books**

1. Willard, Merritt, Dean, Settle.(1989). =*Instrumental Methods of Analysis*. 7th ed. ACS publications

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## DEPARTMENT OF CHEMISTRY

**SUBJECT NAME: INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS-PRACTICALS SUBJECT CODE: 16CHU514A**

**SEMESTER: V** **CLASS: III B.Sc CHEMISTRY**

## LECTURE PLAN-PRACTICAL

Total no. of hours: 48

| S.NO | LECTURE DURATION | TOPICS TO BE COVERED | SUPPORT MATERIALS |
|---|---|---|---|
| 1. | 4 | Procedure writing | |
| 2. | 4 | Safety practices in chemistry laboratory | |
| 3. | 4 | Determination of the isoelectric pH of a protein | |
| 4. | 4 | Titration curve of an amino acid | |
| 5. | 4 | Determination of a mixture of cobalt and nickel(UV/Vis spec) | |
| 6. | 4 | Study of electronic transition of organic molecules ( i.e., acetone in water) | |
| 7. | 4 | IR absorption spectra (study of aldehydes and ketones) | |
| 8. | 4 | Quantitative analysis of mixture by gas chromatography (i.e., $CHCl_3$ & $CCl_4$) | |
| 9. | 4 | Nuclear magnetic resonance | |
| 10. | 4 | Detection of pollutants or illegal dumping | |
| 11. | 4 | Viva-voce | |
| 12. | 4 | Model examination | |

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**SUBJECT CODE: 16CHU514A**
**SEMESTER: V** **CLASS: III B.Sc CHEMISTRY**

**Ex. No.: 1**

## SAFETY PRACTICES IN THE CHEMISTRY LABORATORY

### SAFETY RULES

The chemistry laboratory is not a dangerous place to work as long as all necessary precautions are taken seriously. In the following paragraphs, those important precautions are described. Everyone who works and performs experiments in a laboratory must follow these safety rules at all times. Students who do not obey the safety rules will not be allowed to enter and do any type of work in the laboratory and they will be counted as absent. It is the student's responsibility to read carefully all the safety rules before the first meeting of thelab.

**Eye Protection:**

Because the eyes are particularly susceptible to permanent damage by corrosive chemicals as well as flying objects, safety goggles must be worn at all times in the laboratory. Prescription glasses are not recommended since they do not provide a proper side protection. No sunglasses are allowed in the laboratory. Contact lenses have potential hazard because the chemical vapors dissolve in the liquids covering the eye and concentrate behind the lenses. If you have to wear contact lenses consult with your instructor. If possibletry to wear a prescription glasses under your safety goggles.

In case of any accident that a chemical splashes near your eyes, immediately wash your eyes with lots of water and inform your instructor. Especially, when heating a test tube do not point it towards anyone. Always assume that you are the only safe worker in the lab. Work defensively. Never assume that everyone else as safe as you are. Be alert for other's mistakes.

**Cuts and Burns:**

Remember you will be working in a chemistry laboratory and many of the equipment you will be using are made of glass and it is breakable. When inserting glass tubing or thermometers into stoppers, lubricate both the tubing and the hole in the stopper with water. Handle tubing with a piece of towel and push it with a twisting motion. Be very careful when using mercury thermometer. It can be broken easily and may result in mercury contamination. Mercury vapor is an extremely toxic. When you heat a piece of glass it gets hot very quickly and unfortunately hot glass look just like a cold one. Handle glass with tongs. Do not use any cracked or broken glass equipment. It may ruin an experiment and worse, it may cause serious injury. Place any broken glass in the proper waste glass container. Do not throw them into the wastepaper container or regular waste container.

**Poisonous Chemicals:**

All of the chemicals have some degree of health hazard. Never taste any chemicals in the laboratory unless specifically directed to do so. Avoid breathing toxic vapors. When working with volatile chemicals and strong acids and bases use ventilating hoods. If you are asked to smell the odors of a substance do so by wafting a bit of the vapor toward your nose. Do not stick your nose in and inhale vapor directly from the test tube. Always wash your hands before leaving the laboratory. Eating and drinking any type of food are prohibited in the laboratory at all times.

Smoking is not allowed. Anyone who refuses to do so will be forced to leave the laboratory.

**Clothing and Footwear**:

Everyone must wear a lab coat during the lab and no shorts and sandals are allowed. Students who come to lab without proper clotting and shoes will be asked to go back to change his or her clothing. If they do not come on time they will be counted as an absent. Long hair should be securely tied back to avoid the risk of being set on fire. If large amounts of chemicals are spilled on your body, immediately remove the contaminated clothing and use the safety shower if available. Make sure to inform your instructor about the problem. Do not leave your coats and back packs on the benchs because they may be contaminated. No headphones, Walkmans, mp3 players or cell phones are allowed in the lab because they interfere with your ability to hear what is going on in the Lab. Cell phones must be turned off.

**Fire:**

In case of fire or an accident, inform your instructor at once. Note the location of fire extinguishers and, if available, safety showers and safety blankets as soon as you enter the laboratory so that you may use them if needed. Never perform an unauthorized experiment in the laboratory. Never assume that it is not necessary to inform your instructor for small accidents. Notify him/her no matter how slight it is.

**Laboratory Care and Waste Disposal**

Remember that the equipment you use in this laboratory will also be used by many other students. Please leave the equipment and all workspaces as you wish to find them. After the end of the each lab, clean off your work area. Wash your glassware. When weighing any material on the balances, do not weigh directly onto the balance pan. Weigh your material on a piece of weighing paper. The balances are very sensitive instruments and should be treated with great care. If you take more reagents than you need, do not put excess back into the bottle. It may be contaminated. Threat it

as waste and dispose of it accordingly. It is most likely that, during any experiment you will perform, you will generate some waste chemicals and solutions to dispose of. Never put them down the sink unless specifically told to do so by your instructor. There will be inorganic, organic, and solid waste containers in the lab. Dispose of your waste in the appropriate container.

**Ex. No.: 2**

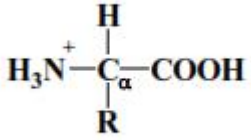
## Titration Curve of an Amino Acid

**Aim:**

A) To determine the titration curve for an amino acid and B) to use this curve to estimate the pKa values of the ionizable groups of the amino acid and the amino acid's pI.

**Introduction**

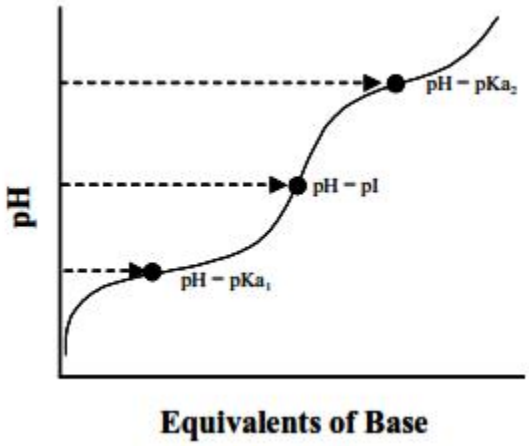
The protein building blocks, amino acids, are polyprotic and have the general structure. The majority of the standard amino acids are diprotic molecules since they have two dissociable protons: one on the alpha amino group and other on the alpha carboxy group. There is no dissociable proton in the R group. This type of amino acid is called a "simple amino acid". A simple amino acid is electrically neutral under physiological conditions.




The order of proton dissociation depends on the acidity of the proton: that which is most acidic (lower pKa) will dissociate first. Consequently, the $H^+$ on the α-COOH group ($pK_{a1}$) will dissociate before that on the α-NH group (pKa).

**Principle:**

The titration curve for this process looks similar to the following:




This curve reveals, in addition to the same information observed with a monoprotic acid, an additional characteristic of polyprotic acids and that is *the pH at which the net charge on the molecule is zero*. This pH defines the **isoelectric point** (**pI**) of the molecule, a useful constant in characterizing and purifying molecules. Using a titration curve, the pI can be empirically determined as the inflection point between the pKa of the anionic and cationic forms. Mathematically, the pI can be determined by taking the average of the pKa for the anionic and cationic forms. The ionic form of the molecule having a net charge of zero is called the *zwitterion*. A few amino acids are classified as triprotic. This is because, in addition to the ionizable protons of the α-COOH and α-NH groups, they also have a dissociable proton in their R group. Although triprotic amino acids can exist as zwitterions, under physiological conditions these amino acids will be charged. If the net charge under physiological conditions is **negative**, the amino acid is classified as an **acidic** amino acid because the R group has a proton that dissociates at a pH significantly below pH 7. The remaining triprotic amino acids are classified as **basic** amino acids due to a) their having a net **positive** charge under physiological conditions and b) an R group dissociable proton with a pKa near or greater than pH 7. Titration curves for triprotic amino acids generate the same information as those for the diprotic amino acids. The pI for a triprotic amino

acid can be determined graphically, although this is somewhat more challenging. Graphical determination, as was the case with the diprotic acids, requires one to know the ionic forms of the amino acid and finding the inflection point between the cationic and anionic forms. Mathematically, the pI foran acidic amino acid is the average of $pKa_1$ and pKa (the pKa of the dissociable proton in the R group); for a basic amino acid, it is the average of $pKa_2$ and

**Method:**

Pipette 10 ml of alanine solution (0.1 M) into a 50 ml breaker. Add 0.5 ml of (0.1 M) HCl from the burette and determine the pH of the solution after each addition. Continue adding acid in until pH falls to about 1.3. Wash the electrode in distilled water titrate a further 10 ml of alanine solution with 0.1M NaOH until pH reaches 12.5.Plot a titration curve for alanine (pH verses titrant in ml).Do the titration of arginine in a similar way and plot their titration curves.

**Result:**

The amount of $Na_2CO_3$ present in the whole of the given solution is =_____ g.

**Ex. No.: 3**

## Potentiometric Titration of Chloride and Iodide Mixtures

**Aim:**

To estimate the amount of sodium hydroxide present in the whole of the given solution using a standard solution of sodium carbonate containing 5.3 g of substance in 1000 ml and an approximately decinormal solutions of hydrochloric acid.

**PRINCIPLE:**

The mixture is titrated with a standard solution of silver nitrate, and the potentiometric end points are indicated with a standard silver-wire electrode – glass electrode pair using a pH-meter for potential measurements. Because the pH during the titration remains essentially constant, the glass electrode's potential remains constant, and this electrode serves as the reference electrode. Thus, this eliminates the necessity of preparing a chloride-free salt bridge for the reference electrode. AgI (Ksp= $1x10^{-16}$) precipitates first since it is less soluble than AgCl (Ksp = 1x10). The AgCl starts precipitating near the equivalence point of iodide titration $[Ag+][Cl^-] = 1x10^{-10}[Ag+]$ at the iodide equivalence point is $(1x\ 10^{-8})^{1/2} = 1*10^{-8}$ M). The potential increment of the iodide titration curve will level off at the point when the chloride starts precipitating, that is, near the iodide equivalence point inflection. This will be followed by the typical S-shaped chloride potentiometric end point. The error in determining the iodide end point is small if it is taken at the point at which the potential levels off.

$$I^- + Ag^+ \rightarrow AgI$$

$$Cl^- + Ag^+ \rightarrow AgCl$$

**SOLUTIONS AND CHEMICALS REQUIRED:**

0.1 M standard $AgNO_3$: Dry the primary standard $AgNO_3$ for 1-2 hours at 110-120C (no longer). Store in a dessicator until it is ready for weighing. Obtain and dry your unknown at 120Cfor 1-2 hours. Store in dessicator until it is ready for weighing.

**PROCEDURE:**

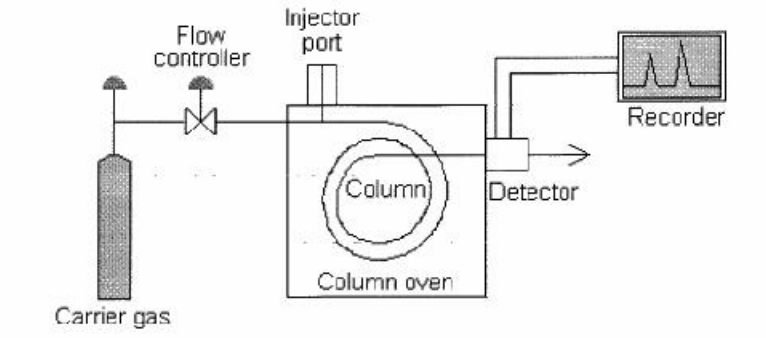
Obtain your unknowns from the instructor and dilute to approximately 150 mL and then put a magnetic stirring bar, and place the beaker on a magnetic stirrer. Immerse the electrodes in the solution, taking care that they do not hit the magnetic stirrer. Connect the silver electrode to the reference terminal of the pH/ion meter and glass electrode to its usual terminal. Stir the solution and titrate the sample with the standard $AgNO_3$. Take "pH" readings (actually pX) at 0.5 Ml increments until a significant increase is observed and then add 0.1 mL increments. After the first end point is reached, add 0.5 mL increments until the second end point is approached and then 0.1 mL increments. Plot the potential versus volume of $AgNO_3$ and determine the end point for the iodide and the chloride. Use these values to estimate the end point for the other two samples and repeat the above procedure for these samples. Titrant may be added rapidly up to within 2 or 3 mL of the end point. Be sure to rinse the electrodes between titrations.

**CALCULATIONS:**

Calculate and report the percent iodide (from the volume required to reach end the first point) and chloride (from the volume required to go from the first end point to the second end point) in your unknown for each portion analyzed.

**Ex. No.: 3**

## Gas Chromatography




In this experiment we will study the method of gas chromatography. Gas chromatography (GC) is one of the most important analytical tools that the chemist has. In this lab you will watch and listen to a video presentation about GC, look at a research-level GC instrument, and learn how to do a qualitative and quantitative GC analysis. A schematic outline of a typical instrument is shown below.

When a sample is injected into the correct column, a carrier gas sweeps the sample through the column. If necessary, an oven heats the system to vaporize the sample and speed its passage through the column. The different components of the sample will be separated by the column When a substance leaves the column, it is sensed by a detector. The detector generates a voltage that is proportional to the amount of the substance. The signal from the detector is then displayed by a chartrec order and/or fed into a computer. Modern gas chromatographs are connected to a computer which displays the peaks of all the substances in the sample. This is called the chromatogram. Software can perform all the calculations you will do in this experiment. An example of an analysis for cholesterol esters is shown above. If it could be done at all, this separation would take weeks by traditional wet chemistry. Here it took 21 min for the analysis and a few hours to prepare the sample for injection into the chromatograph. However, so that you can understand how the computer does its analysis, we will supply you with peaks drawn by a chart recorder and you will

perform all the measurements and calculations. The kind of signal displayed by a chart recorder is more-or-less a triangular shaped peak. This is because the detector signal causes a vertical deflection of the recorder pen at the same time that the chart paper is moving under the pen The time that it takes a substance to pass through the instrument from injection to detection is called the **retention time, $t_r$**. The retention time, t, is measured from the **injection point** to the **peak height**. The peak height is the highest point of the peak and is the only reproducible point on the peak. Since the chart paper moves at a constant speed, the box divisions are proportional to $t_r$ and you can measure t in box divisions for this experiment.

The amount of substance in a sample is proportional to the **area under the peak** of that substance. However, the proportionality constant is different for each substance and detector. Therefore, to do quantitative analysis by gas chromatography, you must first determine the proportionality constant for each substance in the sample. You will do this by constructing calibration lines as described below.

There is another piece of information that you need in order to do quantitative GC analysis. This is the **attenuation (attn)**. Since most signals from the detector are too large for the recorder mechanism to handle, there is a switch on the chromatograph that attenuates or reduces the size of the signal. For example, if the attenuation of a peak is 512, the signal has been reduced 512 times. So you must multiply the area under the peak (the area that you measure on the chart) by 512 to get the true area that is proportional to the original signal. Thus, it is **area x attn** that is proportional to the amount of substance injected. You will be given a packet in lab that contains copies of GC chart recordings of the following four compounds:

There are two kinds of information available on the recordings in the packet.

1. The retention times of each substance as determined from the distance in box units between the injection point and peak of each curve. As stated above, we will use box units for retention times because they are proportional to the time and serve us just as well.

2. The peak areas corresponding to injection of different amounts of each pure substance. You are given a number of peaks (usually 7 or 8) for each pure compound made by varying the quantity (in microliters, µL) injected, and the attenuation setting for each peak.

You are to plot a calibration line for each substance using Vernier's Graphical Analysis program. You will then perform a linear regression on each line to determine the slope and y-intercept of the line. After the calibration lines are plotted and approved by the instructor, you will be given the chromatogram of an unknown mixture to identify. By determining the retention time of each peak of the unknown, you can identify the substance that the peak corresponds to. By measuring the area x attn of each peak, you can use the formula of a straight line ($y = mx + b$) from the calibration line to determine how much of each substance was injected. Determining Retention Times and Preparing Calibration Graphs

On the chromatogram of all the pure substances (the last page of the packet you will be given in lab), measure the retention time of each peak in box units, to the nearest 0.1 box units, and record it directly on the graph near the peak. For each peak, the retention time is measured from the injection point to the top of the peak as shown below. Transfer the values to Data Table – Retention Times.

**Result:**

You will write formal report for this laboratory. For your unknown report, provide your name, your unknown number, the percent ethanol in your unknown sample, the standard deviation. Although this experiment was performed in groups, each student must write his/her own report.

**Ex. No.: 4**

**$^{1}$H NMR**

**Aim:**

To estimate the amount of ferrous sulphate present in the whole of the given solution using a standard solution of Mohr's salt, containing 39.18 g of the substance in 1000 ml and an approximately decinormal solution of potassium permanganate.

## INTRODUCTION

### Nuclear Magnetic Resonance, NMR

This week you will observe molecules interacting with radio-frequency energy in a technique called nuclear magnetic resonance, NMR. The amount of structural information that can be deduced from NMR spectra is un rivaled by other methods. When placed in a magnetic field, the nuclei of many atoms exhibit multiple states, and will absorb or emit energy when changing from one state to another. The energy difference between two of these nuclear states is detected in NMR. We observe only one isotope at a time because different isotopes absorb in very different portions of the spectrum. Different atoms of the same isotope will absorb slightly different amounts of energy when they are in different environments.

### $^{1}$H NMR:

Now let's switch nuclei and look at $_{1}$H NMR. Figure 2a shows the $_{1}$H NMR spectrum of isopropanol. Remember that we are considering only the hydrogen atoms this time.H NMR is more complicated than $_{13}$C NMR, but rewards us with more information. Just as the number of peaks in theH-NMR tells us the number of types of hydrogen atoms. The spectrum also tells us how many hydrogen atoms of each type are present, and how many hydrogen atoms are on the neighboring carbon atoms.

Look closely at the spectrum in Figure 2a. It shows three peaks, representing the three types of

hydrogen atoms in isopropanol. These peaks aren't as simple as the ones in our C spectrum. The peak at 1.25 ppm is split into two closely spaced peaks; this unit is called a doublet. The peak at 3.95 ppm is split into seven closely spaced peaks; this unit is called a septet. For the moment, ignore this extra splitting. Look at the structure of isopropanol in Figure 2a. You should be able to convince yourself that this structure has three types of hydrogen atoms: six identical hydrogen atoms from the end -CH groups, one hydrogen on the central carbon atom, and one hydrogen on the oxygen. So the three types of hydrogen match up with the spectrum's three peaks, but which peak corresponds to which type of hydrogen? As with the $^{13}C$ NMR spectrum, the positions of the peaks tell us what they hydrogen atoms are attached to. The presence of double bonds and more electronegative elements such as oxygen or halogens moves peaks further to the left. While peak position provides useful information, we will focus on area and splitting to identify peaks.

One way to tell which peak goes with which atoms is to look at the area under the curve for each peak. The integrated area of a peak is proportional to the number of hydrogen atoms it represents. Our structure has six hydrogen atoms of one type, so the peak that corresponds to these hydrogens should have an integrated area six times larger than the other peaks. Just from looking at the spectrum, you can see that the peak at 1.25 is much larger than the others and probably corresponds to the six –CH type hydrogen atoms. The small numbers that appear directly below each peak are the integrated areas of those peaks as measured by the NMR instrument. We make a ratio of these areas and then reduce the ratio. From our spectrum, the areas are 31.7 : 37.7 : 190.1 which reduces to approximately 1:1:6. We now know that the peak at 1.25 ppm is due to six identical hydrogen atoms, so this must be the six -$CH_3$ type hydrogen atoms.

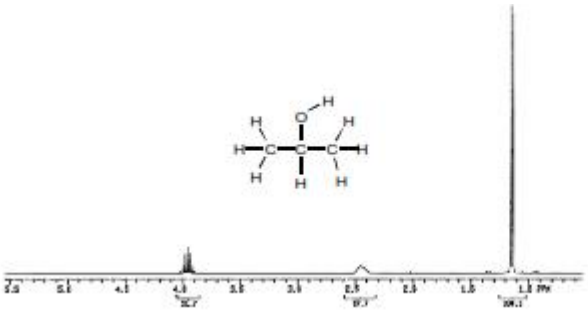
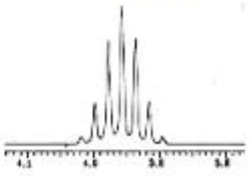


Figure 2b: Expanded Septet



**PROCEDURE**

You will be working with an assigned partner. You should work as a team on all aspects of this experiment and turn in a single lab report for your team. Each person should do the normal pre-lab entry in your own notebook, but once in lab all data will be recorded directly in the data tables at the end of this experiment. Finish your report before leaving lab--staple your report, NMR spectra and pre-lab pages together, and turn this in before leaving lab.

Part 1: Learning about NMR

Large organic molecules are often composed of small, familiar units connected in new ways. By recognizing these small units, we can simplify our understanding of the larger molecules.

Part 2 will include some of these units, so you will use your Part 1analysis to identify your Part 2 unknown. Use the description of the iso-propyl group from the introduction as a guide as you predict the number and type of peaks expected for n-propyl, ethyl, and methyl groups on the data sheet.

Part 2 Obtaining your NMR Spectrum

Before going to the NMR room (Trexler 162) prepare your sample in the regular lab room. Attach a new tip to the automatic pipetter that is set for 40 μL (0.040 mL) and pipet this volume of your

NMR unknown into a clean NMR tube. Record the number of the unknown. Then place the NMR tube in the measuring tube and add the NMR solvent, $CDCl_3$, until the liquid level is between the lines. This places 0.7 to 0.8 mL in the tube, the necessary volume. Be sure to close the stock bottle of $CDCl_3$. Cap, mix, and take this sample to the NMR in room 162 where the instructor wil help you record the $^1H$ NMR spectra.

Part 3 Identifying your NMR Unknown

Follow the directions on the report sheet pages.

**Summary:**

NMR help chemists learn about a molecule's structure. Think about how the information we get about structure from each is similar and different. Some of the techniques chemists use are simple and fast but provide limited information. Other techniques are harder or longer but provide more information.