JoVE: Science Education

Sample Preparation

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Chemistry Science Education Title:

Sample Preparation

Overview: Sample preparation is the way a sample is treated to prepare it for analysis. Careful sample preparation is critical in analytical chemistry to accurately prepare either a standard or unknown sample for a chemical measurement. Errors in analytical chemistry methods are categorized as random or systematic. Random errors are errors due to change and are often due to noise in instrument. Systematic errors are due to investigator or instrumental bias which introduces an offset in the measured value. Errors in sample preparation are systematic errors, which can then ~~Errors in sample preparation of standards can~~ be propagated to cause uncertainty or inaccuracies through improper calibration curves. Systematic errors can be eliminated through correct sample preparation and proper use of the instrument. Poor sample preparation can also sometimes cause~~Other problems with sample preparation can cause issues with the measurement itself, making it inaccurate or causing~~ harm to the instrument.

To make a solution, one must consider the solubility of the substance that is being measured. The compound of interest must dissolve in the solvent in order to make a solution. Solubility is a factor of intermolecular interactions of the analyte with the solvent and can often be manipulated by changing the type of the solvent or the pH.

The first step to making a sample is ~~generally~~ choosing proper glassware and making a solution. Most samples in the liquid phase are made in volumetric flasks. Volumetric flasks are made to contain a certain volume of liquid at a given temperature (normally 20 °C), and are calibrated to be accurate less than 0.02% if they are class A glassware. Volumetric flasks are much more accurate for measuring liquids than graduated cylinders.

To make a solution of a solid, the solid must first be accurately massed~~. A calibrated scale should be used~~with a calibrated scale. However, the mass of some reagents and precipitates can change because they are hygroscopic and adsorb water. If the reagent has adsorbed water, it is impossible to use the non-hydrated molecular weight to obtain the correct number of moles. To remove adsorbed water, solids that are thermally stable ~~can be~~ are dried in an oven at around 110 °C. Solid reagents and precipitates ~~should~~are then ~~be~~ stored in a desiccator~~, which contains~~ containing a desiccant that ~~will~~ adsorbs any water present.

If the sample to be diluted is a liquid, a ~~To add a liquid to a solution,~~ pipette~~s~~ is~~are~~ normally used to measure it. A glass transfer pipette is typically calibrated to deliver one accurate volume and the last drop stays in the pipette and should not be blown out. A measuring pipette will have multiple markings on it, similar to a burette, and is less accurate but more versatile than a transfer pipette. Smaller volumes can be measured using variable micropipetters, with disposable plastic tips, and these are available in volumes from 1-

Commented [ASW1]: The organization of the first two sections is a bit disjointed. Arrange in the order that the steps would be carried out in. (I doubt one sample prep would require all of these, but just the overall order).

Commented [ASW2]: Briefly cover random vs. systematic error: Where they come from and how they affect your experiment.

Commented [JV3]: I wasn't sure how to split the overview and principles material in this video. If you want all the solubility background from principles, it could be moved here.

5,000 µL~~ul~~. Micropipetters should be calibrated every 6 months in order for them to maintain accuracy. If plastic is an issue, small microsyringes can also be used to measure out volumes in the microliter range.

~~To make a solution, one must consider the solubility of the substance that is being measured. The compound of interest must dissolve in the solvent in order to make a solution. Solubility is a factor of intermolecular interactions of the analyte with the solvent and can often be manipulated by changing the type of the solvent or the pH.~~

After a solution is made, there are other elements of sample preparation that may be pertinent. Any sample with solid remaining in the liquid should be filtered. Traditional filtration uses a set-up with a filter paper that sits in a fritted glass funnel on top of a filter flask with an arm where vacuum can be pulled. This type of filtering is used to collect a precipitate in experiments such as gravimetric analysis. Smaller samples that are to be analyzed can be cleaned up via syringe filtering, where the sample is loaded in a syringe and then passed through a polymer filter with down to 0.2-nm resolution. Additionally, spin filters are available, where the sample is loaded in a microcentrifuge tube with a filter, the tube is placed in a centrifuge, and the filtered liquid is at the bottom after centrifugation. Spin filters are also used to concentrate larger analytes, such as proteins. Syringe and spin filters are useful to filter out contaminants and other solids that might interfere with the instrument or measurement. The type of filtration used depends on the amount of sample and the size of the solid that needs to be filtered out.

~~Reagents and precipitates can be hygroscopic and adsorb water. If this happens, the mass of the solid changes, which can lead to inaccuracies in weighing massing out the correct number of moles. Solids that are thermally stable can be dried in an oven at around 110 °C. Solid reagents and precipitates should then be stored in a desiccator, which contains a desiccant that will adsorb any water present.~~

Principles:

Solubility is the amount of substance that will dissolve in a liquid. Generally, if less than 0.1 g will dissolve in 100 mL of solvent, a substance is considered to be insoluble. Solubility depends on intermolecular interactions with the analyte and thus, the general rule in solubility is “like dissolves like”. Thus, polar substances tend to dissolve well in polar solvents while non-polar analytes dissolve well in non-polar solvents. Solubility of solids in a liquid is generally greater at higher temperatures, because of the added energy and molecular motion.

Chelation is accomplished by multidentate ligands that have multiple binding sites for a molecule. The most common chelating agent for metal ions is ethylenediaminetetraacetic acid (EDTA), which is hexadentate and binds through 2 nitrogen and 4 oxygen atoms. It has 6 acidic protons that it can lose upon metal-EDTA complex formation. The formation constant for binding is pH specific and the pH is often adjusted to adjust the specificity of the chelation reaction.

Because EDTA can complex with many different metals, masking is needed in order to perform analysis of a specific metal. Before the addition of the chelating agent, a masking

agent is added to protect the ion of interest from reacting with the EDTA. The formation constant for the masking agent-metal complex must be greater than the formation constant for the EDTA-metal complex so that the EDTA will not react. For example, fluoride masks Al^{3+} and Fe^{3+}. Cyanide is another common masking agent that does not react with Mg^{2+}, Ca^{2+}, or Pb^{2+} but does react with other metals such as Cd^{2+}, Hg^{2+}, Fe^{2+}, Fe^{3+}, and Ni^{+}. Cyanide can form a toxic gas at low pH so it should always be used in a solution above pH 11. Demasking releases the masked metal ion; for example cyanide can be demasked by a chemical reaction with formaldehyde. Masking and demasking allow selectivity for measuring components of complex mixtures.

Procedure:

1. Making a Ssolution from a solid: Gglassware and Ssolubility.

1.1. Choose the correct glassware to make solution.

1.2. Clean the glassware thoroughly via an acid bath of 1-% HCl or HNO_3 along with soap to remove any impurities (safety warning: with any strong acid, use gloves, goggles, and other appropriate personal protective equipment).

1.3. Rinse the glassware several times with distilled water. Dry in an oven if needed.

1.3.1.4. To make a solution from a solid, mass out the correct amount of solid.

1.4.1.5. To make a solution of a solid in a volumetric flask, pPut the solid in first the volumetric flask and then fill about ¾ full with solvent.

1.5.1.6. Swirl to fully dissolve the solid before you filling the volumetric flask fully.

1.7. Fill the volumetric flask to the line. The meniscus should just touch the fill line. Then invert the flask several times with the cap on to further mix if necessary.

2. Making a Solution from a liquid.

1.6. Choose the correct glassware to make solution.

2.2. Release the liquid into the proper volumetric flask for making the solution. Do not blow out the last drop.

1.9.2.3. Fill the volumetric flask to the line so the meniscus touches the line. Mix the solution by inverting several times.

2.3. Filtering

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Commented [ASW4]: These last two steps don't flow from the previous 6. Flush them out into their own mini-section, where you use a transfer pipette to make a solution.

~~2.1.~~3.1. For a filter flask set up, place a piece of filter paper on the fritted glass filter.

~~2.2.~~3.2. Attach the fritted glass filter to a filter flask.

~~2.3.~~3.3. Attach a vacuum to the arm of the filter flask. A trap can also be used to prevent any liquid from going into the vacuum.

~~2.4.~~3.4. Turn on the vacuum and pour the sample through the filter paper.

~~2.5.~~3.5. Filter until a dry powder is left. Continue to dry the sample in an oven if a dry precipitate is desired.

~~2.6.~~3.6. To syringe filter, add the sample to a clean syringe with a Luer lock end.

~~2.7.~~3.7. Screw the syringe filter into the Luer lock. Push the plunger on the syringe and collect the liquid after the filter.

~~2.8.~~3.8. For a spin filter, pre-rinse the filter with buffer or ultrapure water.

~~2.9.~~3.9. Insert the spin filter into a microcentrifuge tube.

~~2.10.~~3.10. Load the sample on top of the filter and cap the tube.

~~2.11.~~3.11. Put the tube into a centrifuge, making sure to balance it properly with another tube on the other side, and centrifuge for 10-30 min, depending on the type of spin filter.

~~2.12.~~3.12. Remove the filter and the liquid in the bottom is the filtered solution.

~~2.13.~~3.13. If the sample cannot go through the membrane, such as a big protein, it will remain at the top of the filter. In this case, turn the filter over, put it in a new tube and spin again. This will produce a concentrated sample.

~~3.~~4. Masking and Chelating

~~3.1.~~4.1. For masking and chelating, adjust the pH to an appropriate value depending on the formation constants of the masking agent and the chelating agent.

~~3.2.~~4.2. Add the masking agent to the solution and allow it to react for at least 10 minutes with the metal ion of choice.

Commented [ASW5]: What metal ion, masking agent, and demasking agent will you be using?

Commented [JV6]: Likely hydrogen cyanide for masking, adding in formaldehyde for demasking. This has to be done at high pH (to avoid making cyanide gas!)but is the most common masking reaction. You can mask Fe2+ and Cu+ in water, for example, with cyanide and the determine the Ca2+ and Mg2+ concentration.

Commented [ASW7]: How long is advisable to wait?

~~3.3.~~4.3. Add the chelating reagent. For EDTA, it typically forms a 1:1 complex the metal ion, so add as many moles of EDTA as metal that will be chelated.

After chelation, demask by adding a chemical that will react with the masked metal ion. The masked substance can then be analyzed or recovered by precipitation.

~~4. Results: Sample preparation~~

5. **Applications:** Spin filters are often used in biological analyses to clean up samples. If cellular debris from cell lysis is a problem, then the sample can be spin filtered and the filtrate at the bottom will be free from particles. If you wish to concentrate a protein or other bigger analyte, then a filter with a small pore membrane can be used that the protein cannot pass through. After spin filtering, the smaller molecules will be in the filtrate at the bottom and will be discarded. When the filter is inverted and spun again in another tube, it can be released from the filter and collected in a concentrated form. Syringe filters are often used to remove dust particles and other small particles from chromatography samples, as the particles could clog the column and cause problems with the instrument.

EDTA is often used for titrations to determine metal contents. The number of moles of EDTA added equals the number of moles of metal. Chelation is also used for extractions in trace metal analysis. Chelating a metal will neutralize the charge and allow it to be extracted into an organic solvent if the chelating agent has a hydrophobic group. Masking prevents a metal from being chelated and therefore from being extracted. This method can be used for sample clean-up or preconcentration of trace metals.

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Commented [ASW8]: Are there any common steps at this point? Are any of the components ever recovered from solution, or is it always only for analysis?

Commented [JV9]: Typically, it is masked for analysis. On occasion, you might do a precipitation and recover it.

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